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CLINICAL AND EXPERIMENTAL

MICROSCOPICALLY DEMONSTRABLE FAT IN NORMAL HUMAN HEART MUSCLE*

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IN June, 1922, two of us worked at New York Hospital[1] in an endeavor to correlate the gross and microscopic condition of the heart as obtained at autopsy with the electrocardiograph curves taken just before death. One of the stains which was employed was scharlach r and we found that minute red droplets appeared in practically every heart that we examined. We were unable to determine whether this indicated fatty degeneration or whether it was a normal finding. In order to decide this point, we obtained hearts from individuals who had met sudden violent death by bullet or stab wounds, etc., and who at autopsy showed no abnormality that was apparent to the unaided eye.

Thirteen hearts were examined from individuals ranging from eight to fifty-six years, the average age being thirty-two years. Sections were taken from each ventricle, septum, and auricle. The tissue was fixed in formalin and within twenty-four hours frozen sections were stained with a saturated solution of scharlach r dye in equal parts of 70 per cent alcohol and pure acetone. The technic followed was that given by Mallory and Wright.[2] At the conclusion of the experiments we stained one of the hearts with osmic acid (formalized material placed in Marchi's fluid and cut by the freezing microtome). The results obtained were similar to those with scharlach r. The table and photomicrographs describe our findings. In regard to the scharlach r we found small red droplets of varying sizes in the sarcoplasm of the cardiac muscle cells. These were arranged in longitudinal and transverse rows,

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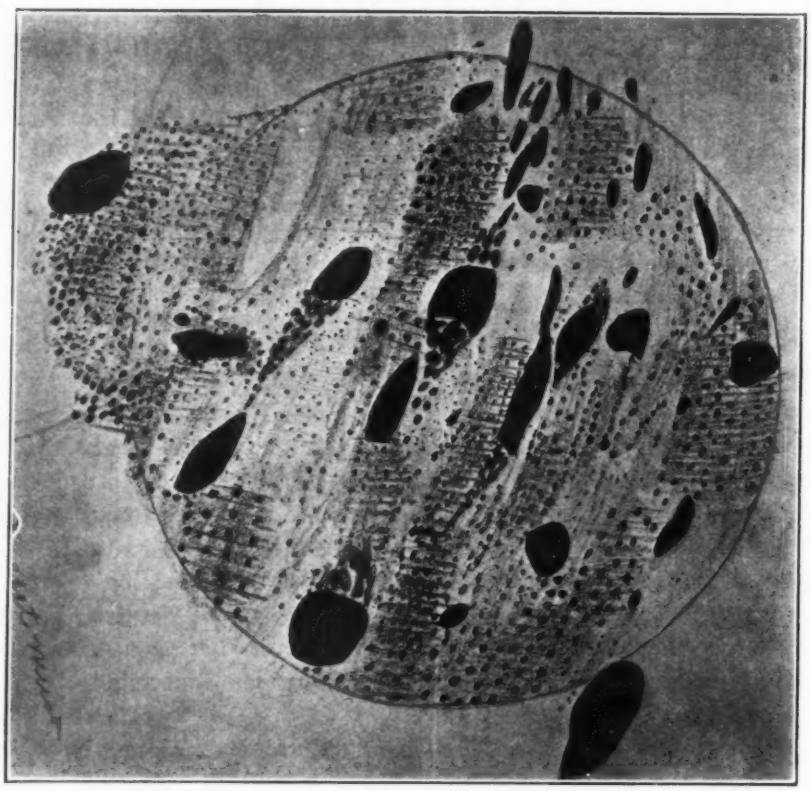
the longitudinal droplets appearing between myofibrillae. The number of granules varied. Some hearts were stained diffusely and uniformly, some scarcely at all, and commonly there were groups of cells which took the stain well, while in the immediate vicinity were cells which contained little, if any, fat.

The so-called pigment of brown atrophy, which is located at the poles of the nucleus and is supposed to be an indication of senility of the muscle, was stained yellow, yellow brown, or golden brown with scharlach r. At first I found it difficult to distinguish between it and the diffuse fat droplets, but



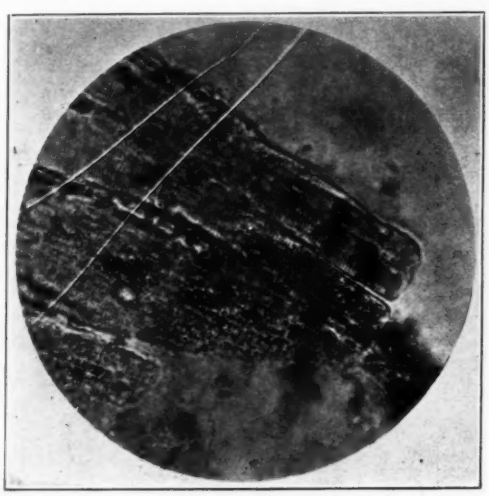
Case A.—Scharlach r stain. Carmine red fat droplets (black in half-tone) in longitudinal rows between the muscle cell fibrillae and arranged transversely (on either side of Krause's membrane). Pigment of brown atrophy at poles of nuclei.

after some experience I was able in practically every case to differentiate between the coarser brownish bipolar granules and the smaller red fat droplets distributed diffusely throughout the cell and arranged in longitudinal and trahsverse rows. With osmic acid the bipolar pigment was stained light brown, whereas the fat granules were intensely black.

Hofbauer,[3] in 1905, described visible fat in normal human fetal heart muscle. Bell,[4] in 1912, first showed that visible fat is normally present in the cardiac muscle of the common laboratory mammals. He also demonstrated that the quantity of visible fat is increased when fatty foods are given and

diminished when the animals are starved. Wegelin[5] found fat in the cardiac tissue of rats. He also examined the heart of an insane man who had jumped out of the window and found fat in quantities, although the organs appeared to be normal at autopsy. He expressed the belief that fat could be demonstrated microscopically in normal human heart muscle. Eyselein[6] of Berlin did not agree with this.

H. Hays Bullard,[7] in 1912, stated that although scharlach r was not specific for neutral fat (scharlach r and sudan III stain neutral fats, fatty acids, soaps and lipoids with varying degrees of intensity) and although he did not believe that all the colored droplets in mammalian cardiac muscle were neutral fat, yet he thought that most of them undoubtedly were. He showed that these bodies were completely dissolved by absolute alcohol and other fat solvents, that they stained characteristically with scharlach r, and red (not blue) with Nile blue sulphate and Nile blue chlorhydrate. The red granules



Case A.—(Ventricular septum.) Scharlach r stain. Carmine red fat droplets (black in microphotograph) in longitudinal rows between the muscle cell fibrillae and arranged transversely (on either side of Krause's membrane).

did not stain with basic analine dyes and were not rendered insoluble by potassium bichromate. These and other properties made it appear certain to Bullard that the droplets were neutral fat and not phospholipines, cholesterin-esters, etc., which may occur normally or abnormally in cardiac muscle.

In a subsequent communication, Bullard[8] showed that there is microscopically demonstrable fat in the normal cardiac tissue of rats, cats, dogs, hogs, oxen and sheep. More than 200 animals were investigated. The fat droplets in the sarcoplasm were arranged in rows between the muscle fibrillae and in transverse lines in segment J on either side of the membrane of Krause. He also noted fatty fibers side by side with nonfatty areas. However, in other cases all the cells showed a uniform diffuse mottled appearance.

Bullard gave conclusive reasons for believing that visible droplets of neutral fat occur in physiologic circumstances in the cardiac muscle fibers of mammals. I quote verbatim:

MICROSCOPICALLY VISIBLE FAT IN NORMAL HUMAN HEART MUSCLE

CASE NO.	AGE	SEX	COLOR	CAUSE OF DEATH	STATE OF NUTRITION AT AUTOPSY	PRESENCE OF DIFFUSE RED FAT DROPLETS IN THE MUSCLE CELL BETWEEN THE FIBRILLAE ARRANGED IN LONGITUDINAL AND TRANSVERSE ROWS	PRESENCE OF GOLDEN BROWN PIGMENT AT THE POLES OF THE NUCLEI, I.E., PIGMENT OF BROWN ATROPHY
A	33	Male	White	Blackjacked i.e. skull fracture, died within few hours	Good	Large quantity	Large quantity
B	45	Male	White	Stab-wound of heart. Died immediately.	Good	Slight or moderate amount	Large quantity
C	45	Male	White	Fracture cervical vertebrae. Died in few hours	Good	Present in some areas	Large, coarse, brown, bipolar pigment
D	23	Female	White	Cerebral hemorrhage died either immediately or within few hours	Very good	Abundance of granules	Good many granules
E	40	Male	White	Fracture of skull. Died in 3½ hrs.	Good	Abundance of granules	Good many granules
F	31	Female	White	Clinical diagnosis not made	Somewhat emaciated	Abundance of granules	Good many granules
G	20	Male	White	Shot to death. Died within 1 hr.	Good	Very few granules	Good many granules
H	42	Male	White	Acute alcoholism	Good	Moderate amount	Very many granules
I	32	Female	Black	Died in few hrs. from hemorrhage after attempted criminal abortion	Good	Moderate amount	Very many granules
J	34	Female	Black	Acute alcoholism	Good	Great many granules	Very many granules
K	56	Male	White	Cerebral hemorrhage from blow	Good	Great many granules	Very many granules
L	15	Male	White	Shot to death. Died instantly	Good	Many granules	Moderate amount
M	8	Male	White	Run over by truck. Died in few hrs.	Good	Many granules	Moderate amount

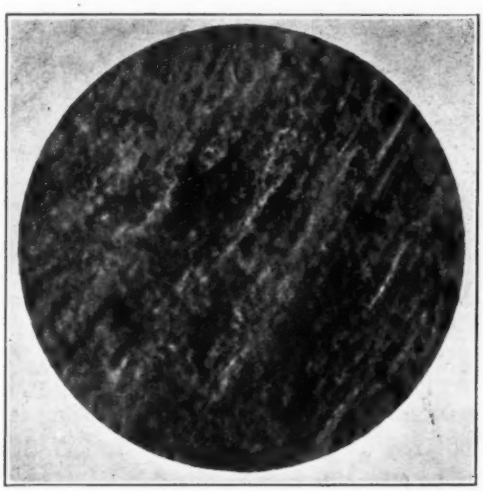
(1) "In each of several well-known species here studied, visible fat is found with great regularity in the heart muscle of all individuals, whether fetal, young or adult. Very few, if any, of the cardiac fibers are absolutely free.

(2) "The fat droplets are not found in the contractile elements. They occupy a definite position in the sacroplasm and do not interrupt the continuity of Krause's membrane.

(3) "There are found in the muscle fibers neither degenerative changes nor any other evidence of any pathological alteration of structure.

(4) "There is no evidence of functional disturbance of the cardiac muscle which contains these droplets.

(5) "The quantity of fat is variable, is decreased in inanition and increased by fatty foods."



Case D.—Osmic acid stain. Results similar to scharlach r. (See Case A.)

Bullard states that the wide divergence of opinion in regard to the fat content of normal cells is due to differences of technic employed. He declares that formalin as a fixative is not always to be relied upon; in fact, fresh sections may be loaded with droplets, whereas formalin fixed tissue contains little fat.

He utilizes a 20 per cent solution of formalin[9] rendered isotonic with 0.75 gm. NaCl per 100 c.c. liquid. Tissues are fixed for one-half to five hours and then cut on the freezing microtome. If this procedure is followed, the quantity of fat does not differ from that obtained in fresh tissues. By means of Herxheimer's alkaline alcoholic solution of scharlach r, fat may often be demonstrated in larger amounts than by the simple alcoholic solution of the dye. (This stain[10] is a saturated solution of scharlach r in 70 per cent alcohol to which 2 gm. of NaOH are added to every 100 c.c. of fluid. Precipitates must be avoided.)

Bullard examined a number of human hearts which were normal in color and showed "no cloudiness, opacity, or yellowish white appearance." He found a moderate amount of fat arranged in a way similar to that demonstrated in animals. However, whether these were normal human hearts, one cannot say.

Within the last few years several articles[11] have appeared in the literature that tend to prove that the pigment of brown atrophy is an endogenous melanin and that some of the red droplets in the cell brought out by scharlach

Case J.—Scharlach r stain. Fat droplets in longitudinal rows between the muscle fibers and in transverse alignment on either side of Krause's membrane.

r are of an exogenous lipochrome. But this lipochrome is stained a deep blue by Nile blue stain, not red as the fat granules are. Other writers, too, state that the pigment can be separated from the fat.

SUMMARY

In human cardiac muscle microscopically visible fat is normally present. The fat resides in the sarcoplasm between the muscle fibrils, and is arranged in longitudinal and transverse rows. Apparently, the amount has no relation to the state of nutrition at the time of death; neither has the age of the individual (eight to fifty-six years), the color, nor the sex.

The picture presented by the diffuse red fat droplets (scharlach r) or the black granules (osmic acid) closely resembled the classical illustrations that many of the textbooks[12] on pathology utilize to depict fatty degeneration of the heart. True fatty degeneration, e.g., the "tiger heart," is easily recognized. Generally the number and size of the granules differentiates the two conditions.

In all experimental work in which sections of cardiac tissue are stained for fat, normal conditions should be kept in mind and controls performed whenever possible. In the investigation of twelve pathologic hearts we found

only one organ demonstrating fatty degeneration. Another writer[13] performing work similar to ours reported "marked fatty degeneration" in every case.

Pathologists were shown our scharlach r sections of normal hearts; without knowing the history of the cases, they stated that the hearts were pathologic, i.e., that they were in the condition of fatty degeneration. They were surprised to learn that the sections were from healthy human hearts.

This investigation illustrates the necessity for experimental investigation of normal human tissues. Pathologic conditions are in many instances known far better than the ordinary healthy state.

The so-called pigment of brown atrophy of the heart, which is supposed to be an indication of degenerative processes, e.g., senility, was found in moderate amounts in two healthy boys, one eight years old, and the other fifteen.

(I wish to express my sincere thanks to Drs. Nonidez and Papinicolau of Cornell University Medical College for their complete cooperation and suggestions and to Mr. H. Wittner who drew the plate.)

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TYPICAL FALL SURVEYS OF HAY-FEVER FLORA

By O. C. Durham, Kansas City, Mo.

GENERAL botanical works are too voluminous and too technical to be of practical use to the busy physician. A few excellent exhaustive hay-fever surveys have been made but they have served outside their immediate districts only as models. It is increasingly evident that local work must be done in every city of any size, but so far such work has been quite expensive. The accompanying table is the summary of the most important data obtained in a number of centers of population visited in one autumn. It is hoped that this report may point the way to effective pioneering in many places. There is always the temptation in this work to list every wind pollinated species encountered, but with the present mass of clinical data it is much wiser for the average physician to know and deal with a dozen locally important plants than to try to apply a mass of half reliable information.

By spending a few days in each place I was able to take physicians into the field and actually introduce them to their common important but often inconspicuous hay-fever weeds. Most of the work was done in waste areas in or very near the cities. The point will bear repetition that the cities are not only the weed breeders but also the residence of the great majority of pollen victims. In some of the places good work has already been done by local men but to make the paper as useful as possible my own observations are recorded.

In the chart only five groups are included. It has been found practical both in field and clinic to work with botanical groups. While specificity in test and treatment is highly desirable, many times group knowledge or grouping has saved the day. Trees are omitted on account of their relative unimportance. Cottonwood, oak, walnut and hickory are found in most of the cities listed and cause an occasional case. Mountain cedar is a real menace in the vicinity of Austin, Texas.

The grass data is not as complete as it should be for spring work. Bermuda grass and Johnson grass are abundant south of Kansas. Sudan grass is widely planted for hay and produces pollen much more freely than its close relative, Johnson grass. All three have long seasons of bloom especially in the south. Various species of Andropogon are widely distributed fall pollinators but their allergic importance has not been established. The composites, including goldenrod, sunflower (Helianthus spp), marsh marigold, broomweed and sneezeweed (Helenium) are common throughout the district visited but their clinical importance is small.

On account of their great importance the ragweeds should always be carefully noted. Short ragweed requires more moisture than false ragweed and western ragweed but less than giant ragweed, cocklebur, or marsh elder. The latter

will grow only on rich black lowland. Narrow-leaved marsh elder needs little water. Burweed marsh elder is the largest pollen producer of its genus but is not found south of Kansas City. It is common in eastern Colorado, Nebraska, and as far east as Chicago. Cocklebur should not be neglected.

DISTRIBUTION CHART
FALL SURVEYS

		St. Louis	Kansas City	Joplin	Wichita, Kans.	Denver	Oklahoma City	Dallas Texas	Austin, Texas	San Antonio	New Orleans	Shreveport, La.
RAGWEED GROUP												
Short Ragweed	Ambrosia elatior	*	*	*	*	*	*	*	*	*	*	*
Giant Ragweed	Ambrosia trifida	*	*	*	*	*	*	*	*	*	*	*
Western Ragweed	A. psilostachya	*	*		*	*	*	*	*			*
Southern Ragweed	A. bidentata	*		*								
Cocklebur	Xanthium commune	*	*	*	*	*	*	*	*	*	*	*
Marsh Elder	Iva ciliata	*	*	*	*		*	*	*		*	*
Burweed Marsh Elder	Iva xanthiifolia		*			*						
Narrow-leaved M. E.	Iva angustifolia							*				
False Ragweed	Franseria acanthicarpa					*						
Bur Ragweed	Franseria tenuifolia								*	*		
CARELESSWEED GROUP												
Redroot Pigweed	Amaranthus retroflexus	*	*	*	*	*	*	*				*
Spiny Amaranth	Amaranthus spinosus	*	*				*	*			*	*
Palmer's Amaranth	Amaranthus palmeri							*	*	*		
Water Hemp	Acnida tamariscina	*	*		*		*	*	*			
GOOSEFOOT GROUP												
Lamb's Quarter	Chenopodium album	*	*	*	*	*	*	*	*			*
Russian Thistle	Salsola pestifer	*	*		*	*	*					
Burning Bush	Kochia scoparia					*						
Shad Scale	Atriplex canescens					*						
WORMWOOD GROUP												
Annual Wormwood	Artemisia annua		*									
Mugwort	Artemisia ludovic. et spp		*		*	*	*	*				
Pasture Sage	Artemisia frigida					*						
GRASS GROUP												
Blue Grass	Poa pratensis	*	*	*	*	*						
Orchard Grass	Dactylis glomerata	*	*		*	*						
Timothy	Phleum pratense	*	*		*							
Redtop	Agrostis palustris	*	*									
Bermuda Grass	Capriola dactylon			*	*		*	*	*	*	*	*
Johnson Grass	Holcus sorg. halapensis			*	*		*	*	*	*	*	*
Sudan Grass	Holcus sorg. sudanensis	*	*		*		*	*		*		

Careless weeds are found wherever there has been cultivation, spiny amaranth demanding very rich soil and water hemp very moist soil. The latter and Palmer's amaranth bear their pollen on a separate plant from that producing seed, while the ragweeds have flowers of both sexes on the same plant.

Lamb's-quarter is not as plentiful in Texas as in Kansas and Missouri and only when it has the advantages of cultivated land and plenty of moisture does it produce pollen abundantly. Russian thistle, *Kochia,* and *Atriplex* are not common except in Colorado where they rival the ragweeds in importance.

The wormwoods are dry prairie plants and while not abundant except in

the west of our district are quite toxic and should be taken into consideration wherever found.

The expense of these surveys has been largely met by Drs. W. W. Duke, Kansas City and R. B. Giles, Dallas. Thanks are due to the following men for their part in helping to make the work possible; Dr. R. M. Balyeat, Okla-

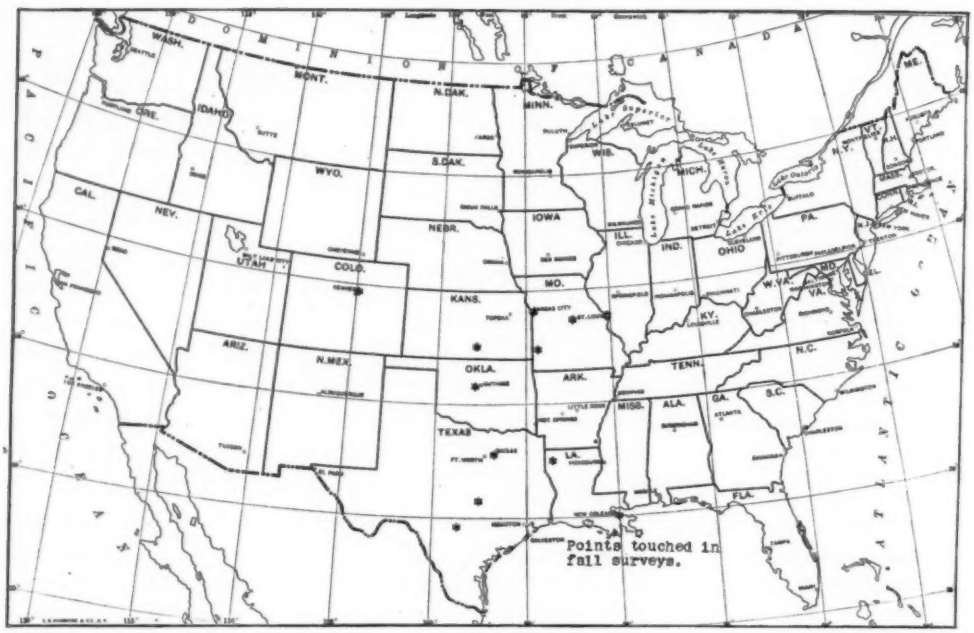

Chart I.

homa City, Dr. T. C. Terrell, Fort Worth, Dr. Chas. H. Eyermann, St. Louis, and Dr. W. C. Tennery, Waxahachie, Texas. Valuable botanical assistance was rendered by Mr. B. F. Bush, Courtney, Mo., Professor B. C. Tharp, Austin, Mr. Albert Ruth, Fort Worth, and Dr. Greenman, Missouri Botanical Gardens. The New Orleans data agrees with that published by Dr. Wm. Scheppegrell and is included merely for comparison.

THE LABORATORY IN DIAGNOSIS AND TREATMENT*

By Ward Burdick, M.D., Denver, Colorado

THE age in which we live is marked by greater advancement in the arts and sciences than in all previous time. Medicine has kept the pace with progress in other departments of knowledge. Fifty years ago the world was practically in darkness as to the nature of the influences responsible for disease in the animal body, as well as the changes wrought in the tissues by these influences. Today the light of knowledge emanates from the scientific laboratories, and medicine, no longer a field of mystery, darkened by superstition, is so illuminated by the achievements of scientific research, that the practitioner of medicine of today is scarcely recognizable as the successor of the family doctor of yesterday.

The influence given to the practice of medicine from the scientific laboratories, has resulted in so broadening its scope, that graduates of medical colleges have ceased to follow the beaten path of precedent; but, like the fiery balls of a rocket, assume a separate course directed to the several fields of medicine, including surgery, urology, otolaryngology, internal medicine, tuberculosis, dermatology, neurology, pediatrics, etc.

The department of medicine which has received the distinction of clinical pathology embodies the practical application of the principles of gross morbid anatomy, pathologic histology, bacteriology, clinical microscopy, serology and chemistry. It, therefore, follows that there has developed a specialty in close relationship to the others and to which they turn, as to a mecca, for guidance. This branch penetrates to the recesses of all departments of medicine, and it is the purpose of this paper to review the various laboratory methods which are available as aids in diagnosis and treatment to the different phases of disease.

In order to relieve this presentation from the personal as much as possible, statements were solicited from physicians and surgeons who are prominent in their respective specialties. Thus, from the field of surgery come the views of Wm. J. Mayo,[1] who says: "As a general surgeon, I am glad to have this opportunity to express my obligation to the clinical pathologists, who have made possible one of the greatest advances in the surgery of the last decade. In the earlier days, the general surgeon, with the aid of the special senses, especially those of sight and touch, was engaged in gross surgery, usually of the destructive type, owing to the fact that the pathologic processes, of which

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[1]Mayo, Wm. J.: Rochester, Minn. An address delivered at the banquet of the American Society of Clinical Pathologists, Rochester, Minn., June 6, 1924.

he was able to take cognizance, were advanced and, as a rule, indicated radical treatment. Today, through the aid of the various clinical laboratories, the surgeon works with the eye of the microscope, if one may so speak. Pathologic processes are discovered in the early stages of development, with the result that operative procedures can be followed when deviation from the normal physiology is least gross, and surgery has become constructive in its general tendency. The surgeon can be regarded as a means of mechanical therapy guided by the microscope, and for this guidance in the clinic we are indebted to MacCarty and Broders and their associates. For example, on opening the abdominal cavity to operate on a neoplasm, a small piece of tissue may be removed for microscopic examination and, within two or three minutes, not only is the nature of the growth known, but also, by the aid of Broders' index of malignancy, based on MacCarty's work on cell differentiation, the prognosis. If the cells are highly differentiated, the prospect of permanent cure is so great that one is justified in undertaking the serious type of operation; whereas, if the cells are largely undifferentiated, the prospect of cure is so small that a formidable operation would not be justified. Again, as in carcinoma of the large intestine or rectum, when enlarged glands occur, instead of assuming that the condition is incurable, a gland may be removed for microscopic examination. Often, enlarged glands are the result of sepsis, in which operation might possibly affect a cure. Not only is the work of the clinical pathologist important in the operating room, but also in the diagnostic clinic. The diagnosis in a given case may be in doubt. Before starting the patient on a long series of indefinite laboratory examinations which he may be in poor condition to withstand, it is often possible, under local anesthesia, to take a speciman of tissue from an enlarged gland in the groin or supraclavicular fossa, a thickened umbilicus, or from some other indicated situation, to be subjected to the acid test of a microscopic examination which tells truly the nature of the trouble and indicates the proper treatment for it."

Regarding the value of clinical pathology as applied to Urology, Davis[2] writes that: "Perhaps no surgical specialty is more dependent upon, or works more intimately with the laboratory, than does urology. The claim of urology to be regarded as a specialty, began with the development of intricate and difficult methods of diagnosis and treatment. The cystoscope, ureter catheter and the microscope have been closely associated in this accomplishment. The clinical pathologist and the roentgenologist have been partners with the urologist in this progress. As a result of this association and cooperation, I believe it may be fairly stated that urology today is one nearly exact art in medicine which latter is notoriously an inexact science. Much of this precision and exactness in urologic diagnosis and treatment is due to the laboratory. No competent genitourinary surgeon, today, would attempt an operation upon a kidney or a prostate, without first obtaining all the available data regarding the kidney and its fellow; or in the case of the prostate, without full knowledge of the combined renal function. The clinical pathologist has assisted in working

[2]Davis, J. B., A.B., M.D., F.A.C.S.: Asst. Prof. of Genitourinary Surgery, University of Colorado School of Medicine.

out the various tests for the determination of these facts—Ambard's constant, the estimation of the freezing point of blood and urine, phloridzin, methylene blue, and finally phenolsulphonephthalein, have been steps in this achievement. Tests of excretion and retention have been perfected. Blood chemistry—the estimation of retained nitrogen, nonprotein and urea, creatinine and other solids in the blood—is essentially a laboratory function. Of no less importance is bacteriology. Animal inoculation for diagnosis, notably in genitourinary tuberculosis, is done for us by the clinical pathologist, and in cases of suspected pyelitis, he confirms the diagnosis and tells us when our patient is cured. The Wassermann and other complement-fixation tests have been of inestimable value in diagnosis and treatment control; but may I here urge more investigation and research in the study of our little friend, the gonococcus. Cultural methods simplified or made more dependable and fermentation or fixation tests more satisfactory, are greatly needed to determine the cure of this widespread disease. In conclusion, however, I believe it may be truly said that the achievement of modern urology is due greatly to the exactness which the clinical pathologist and his laboratory have given to this specialty."

The use of the laboratory in gynecology and obstetrics may be gleaned from the expression of Ingraham,[3] who avers that: "No conscientious obstetrician in this day and age attempts to care for the pregnant woman without frequent routine laboratory examinations. We are dependent on the laboratory for the diagnosis and treatment of the various toxemias in pregnancy, and look to these workers for further advance as to the causes and classification of these not thoroughly understood abnormalities. There are many routine obstetric questions, such as, bacterial determination and tissue examination, which come up constantly. How little we would know of the physiology and the development of the ovum, if it were not for the laboratory. In gynecology, consider not very many years back, how frequent was the use of the curette, and the many diseases of the endometrium described. Through the work of Hitchmann and Alder, the various types of endometritis, have for the most part, been recognized as different stages of the menstrual cycle, and the curette is now seldom used except for diagnosis. The interrelation of the glands of internal secretion is probably more manifest in the study of gynecology and obstetrics than in any other branch of medicine. Much is expected from the clinical pathologist that these observations may be developed along practical lines. The pathologist has crystallized the subject of gynecology, so that today, lesions are recognized and classified and proper treatment instituted. As in every branch of medicine, gynecology and obstetrics are based on a sound knowledge of physiology and pathology for the proper understanding of these subjects."

The clinical pathologist comes in contact with the public only upon invitation by the profession, as it were; his activities are carried on behind the scenes, a fact emphasized by Carmody[4] who reminds us that: "While we are

[3]Ingraham, A. B., Ph.B., M.D.: Prof. of Obstetrics and Gynecology, University of Colorado School of Medicine.

[4]Carmody, T. E., M.D., B.D.S., D.F.C., F.A.C.S.: Past President of American Academy of Ophthalmology and Otolaryngology; Vice President of American Laryngological, Rhinological and Otological Society.

considering the value of different advances in medicine and surgery we most frequently think of what we see in the clinic room as of greatest importance, and while I do not wish to give the idea that marked improvement in technic of operating has not been found valuable, we should remember the man behind the lines as contributing much to the success of the operation. The clinical pathologist in the modern hospital is as necessary to the welfare of the patient as the internist or the surgeon, and many times saves us a false step. From the standpoint of the otolaryngologist the clinical pathologist may give valuable assistance in examining suspicious tumors at the time of operation, when, without his aid the patient might lose his larynx, or, if the growth should be malignant, an extensive operation may save his life. The same may be said of the accessory nasal sinuses. The Wassermann may solve the riddle of a persistent headache. The blood culture may help us to steer a course leading to success in suspected sinus involvement, either before or following a mastoid operation. Spinal puncture and the microscope may hasten interference on one hand or prevent meddlesome surgery on the other. These last-mentioned aids are to a great extent emergency in nature, but we must not forget the every-day routine examinations of blood, urine, and the various secretions and excretions, which may require hours of labor to point the way."

Turning from the surgical specialties to the several branches of internal medicine we quote from Sewall,[5] who writes that: "It seems but yesterday that the medical reactionary ventured to flaunt contempt upon the practical value of laboratory procedures, which now admittedly furnish essential material for the foundation of diagnoses. None will deny that certain examinations, as of the urine and of blood microscopy, should be performed as routine measures; for even superficial tests, if accurate, may be of intrinsic value or may lead to elaborate chemical analysis of the blood or investigation of renal efficiency. The clinical history or status of a case often demands that what may usually be regarded as an exceptional examination, become a routine measure—as the Wassermann test of blood and spinal fluid, a study of the blood pressures, blood grouping, or estimation of basal metabolism. The trained roentgenologist often demands information as to the clinical history of his subject before venturing to make a diagnosis from his x-ray findings. Still more important is it for the competent laboratory pathologist to become cognizant of the clinical status of the person under observation, in order not to lead astray the so-called clinician. The former should always be considered potentially, at least, as a consultant with the latter. The laboratory pathologist is neither a master nor a servant; but, as a matter of fact, we treat him as in the old days physicians were wont to treat the surgeon, as an operator who was bid to cut this or remove that with the aid of his trained technic. It is probable that many a diseased appendix has been allowed to rupture while the clinician, not trusting his own experience and common sense in interpreting indications has vacillated under the laboratory findings of casts and albumin in the urine, and fatally deferred operation until an expert could be found to catheterize the ureters. Frank discussion of the significance of such signs might save many lives. The

[5]Sewall, Henry, M.D., Sc.D., Ph.D.; Professor Emeritus of Medicine, University of Colorado School of Medicine.

state of the patient as seen by the clinician takes precedence over any methodologic indications and the accumulation of 'scientific' data useless to the patient is dangerous pedantry. Besides a vast fund of learning, the laboratory staff should be able to put at our service, technicians meticulously trained in the performance of many procedures, such as the transfusion of blood, spinal puncture, etc. The laboratory man or woman thus comes in direct contact with the patient where applied psychology is of no little moment. The routine examination of tissues excised in the operating room not only may form the basis of valuable scientific deduction, but incidentally, it may serve to curb the operative fury of our colleague in whom the normal appendix excites, as it were, a persecution complex. In brief, it is my belief that, radiating from a narrow basis of routine examinations, the laboratory pathologist should be prepared to execute a far broader range of biologic investigations than is commonly in vogue, while no step should be taken unless the way is lighted by pathologic indications. For the perception of these indications, as in the interpretation of findings, the educated pathologist is an indispensable factor to the welfare of the patient. His duty does not end with the statement of his findings; he should be expected, in all appropriate cases to explain them and to point out their significance. This demands, at times, close study of the patient in cooperation with the clinician."

As is well known, internal medicine is coming to be divided into subspecialties, as for instance the care of diabetics, the tuberculous, etc. From the realm of tuberculosis, Corper[6] expresses himself as follows: "When asked to state my views on the laboratory in diagnosis and treatment in the few words allowed me for this purpose, I feel like the commanding officer who was given one hour's notice to embark for overseas service. The diagnostic laboratory in the clinic and the sanatorium is now so well established that it seems fair to take an inventory of, at least, a few of its important assets. I need not go far into the explanation of the examination of the sputum for tubercle bacilli, for all are well aware of the significance of a positive finding; whether obtained by examining the ordinary smear or by means of some of the more modern concentration methods accurately performed and under precautions to eliminate all possibilities of error. I dare say, however, there are few who fully recognize the value of this method in treatment. Admittedly we are aware of the fact that it is good practice to note whether our case is a persistent open case or one that has become closed, for much of the family's future welfare depends upon this; but it is only in the sanatorium where we can watch our cases year in and year out, that we appreciate all the possibilities in prognosis. We have passed that stage in our preliminary experiences when we believed tuberculosis incurable. It is true there are still some cases that come to us thus, but who wishes to judge absolutely that such is the case and who will not, now and again, change his views on further accurate study? Specifically our patient comes to us, he is at the present time not satisfied to know that he is tuberculous but he wants to know what the future has in store for him, and it is our obligation to give him the prognosis to the best of our

[6]Corper, H. J., M.D., Ph.D.: Director of Research, National Jewish Hospital, Denver.

ability and with the aid of our most modern scientific weapons. Advisedly I call them weapons because too many of us stand off in fear and horror of them. I believe I can best illustrate my point by citing the conclusions of an article by Wingfield[7] and Wilson[8] of the Brompton Hospital Sanatorium, Frimley, in England, which just appeared in a recent number of the *Lancet*. They investigated the criteria for artificial pneumothorax in relation to treatment and state that among other indices the persistence of tubercle bacilli in the sputum after the induction of artificial pneumothorax is a feature of bad omen. Their figures showed that if the sputum remains positive the chances are nineteen to one against the patient being discharged as improved. Likewise we have learned that a great deal of information during treatment and in prognosis is obtained by means of the proper use of the urochromogen or diazo-reactions, repeatedly and conscientiously performed. They are an index of tissue destructive processes going on in the body, and as such, their persistence spells an unfavorable prognosis. I could mention others but refrain from doing so for lack of time. True you may say, but haven't we other means of determining the same points? Yes and no—Were we to give the time and effort, we might obtain the same information by other means, and yet frequently in the experience of the laboratory man, he has had to point out a plausible error on the part of the clinician and surgeon. It is human nature to err, yet our duty to the patient as physicians will not condone this, especially when we are able to make use of simple, economical and rapidly performed tests. An impending intoxication can frequently be discovered by chemical tests before it has reached serious and irremediable proportions. The slogan in tuberculosis which can well be applied to all other phases of medicine is: early correct diagnosis paving the way for early treatment, spells a good prognosis; and in this, the laboratory has developed instruments and methods of precision which are continually being perfected for speed and simplicity, besides being exhaustively evaluated by progressive students of our profession. Shall we stand back and ignore the beacon light of the times, or shall we still further increase the average span of human life, by treating with the appearance of the first sign of a pathologic condition, and when it is still in a remediable stage and can follow its course by means of rapid, delicate and accurate tests to restitution? The laboratory like the sanatorium has, by established ability, come as a permanent fixture; and those of us who do not avail ourselves of and become cognizant of both of these weapons of modern medicine are not fulfilling the requirements and duties placed upon us in accepting this noble profession as a life's work.''

Lingenfelter,[8] speaking with reference to the laboratory in the diagnosis and treatment of diseases of the skin quotes: ''An old and true saying, which is, 'That, the better general practitioner one is, the better specialist he is likely to become.' We could equally well go a step farther and say, 'The more that he

[7]Wingfield, R. G., M.D., M.R.C.P.: Brompton Hospital Sanatorium; and Wilson, G. Selby, M.D., M.R.C.P., D.P.H.: Manchester.

[8]Wingfield, R. G., and Wilson, G. Selby: An Investigation into Criteria for Artificial Pneumothorax Serviceable as Guides to Treatment, Lancet, London, 1924, ccvii, 163.

[8]Lingenfelter, G. P., M.D.: Professor of Dermatology and Syphilology, University of Colorado, School of Medicine.

makes use of the facilities of the laboratory, the greater dermatological success will he enjoy.' It is much to be regretted that in studying the classification of diseases of the skin, one sees so frequently the word 'unknown' in the paragraph devoted to etiology. Particularly is this true of the malignant new growths, of tumors of the lymphatics and of the blood vessels. The laboratory gave us our first accurate knowledge of the cause of syphilis and paved the way to more successful treatment. It is to the laboratory that we must look for the cause and, eventually, the cure of cancer. To this department of medicine we are indebted for the knowledge of the causal agents in those diseases of the skin due to parasites and fungi. In this connection, special mention may be made of epidermophytosis which until within comparatively recent years, had been, all too frequently, wrongly diagnosed and treated. Many of the cases being confused with eczema, pompholyx, and dermatitis venenata. It is extremely possible that many cases went unrecognized. While Hippocrates affirmed that 'The body can only be understood as a whole,' thus hinting against the possible dangers of excessive specialism, we are also reminded of an aphorism of that estimable gentleman, Celsus, who, during the reign of Tiberius Caesar, wrote, 'It is not to be thought that he should know the remedies for disease, who does not know their original causes.' Thus, clinical observation based upon experience and supplemented by accurate laboratory work must eventually lead us to a more exact knowledge of the causes of, and the remedies for, many of the diseases of the skin which are now only partially understood.''

Perhaps no department of medicine requires closer cooperation with the laboratory than that of neurology. This is stressed by Moleen[9] and Bluemel[10] in the following communications. Says Moleen: ''As in other departments in medicine, the laboratory is a most important aid in the determination of the nature and location of nervous disease; but while approaching exactness, is still relative in value and, therefore, should never supersede clinical evidences. The determination of the blood reactions may often decide the presence or absence of inflammatory foci, and the careful quantitative estimation of urinary solids contributes to the establishment of renal insufficiency in cerebral arteriosclerosis and associated uremia. Examination of the spinal fluid may supply evidences of value in the explanation of pressure signs in the brain and spinal cord; and especially in trauma, will careful examination of this fluid often lead to definite conclusions. The most important association of the laboratory and neurology, however, is in that group of diseases which may differ widely in their clinical aspects, and yet, are attributable to the syphilitic virus, even though this virus may, with good reason, be suspected of significant morphologic and biologic variations. It is in the careful examination of the complement-fixation characteristics of the blood and spinal fluid which often makes a definite statement possible with reference to the clinical type of disease as well as to the prognosis. In the evolution of the various tests there has arisen considerable difference of opinion as to the value of each of the groups, such as the phases of Nonne or the

[9]Moleen, Geo. A., Prof. Neurology and Acting Chief of the Dept. of Nervous and Mental Diseases, University of Colorado; Pres., Central Neuropsychiatric Assn.

[10]Bluemel, C. S., M.D., M.A., L.R.C.P., M.R.C.S.: Clinical Instructor Neurology and Psychiatry, Univ. of Colorado, School of Medicine; Editor, Colorado Medicine.

titration formula of Kolmer and others. The painstaking laboratorian has made the gold-sol reaction of Lange of inestimable value, especially in differentiating the vascular from the parenchymatous types of disease. Finally the most significant place for the laboratory is in the intensive treatment of cerebrospinal diseases, and especially syphilis. It is here that closest association between the neurologist and the clinical pathologist be encouraged. The intravenous or intraspinal treatment is best administered by the competent clinical pathologist, who can also supply, at the same time, evidences by means of which the clinician may draw helpful deductions; and thus may guide in the modification, continuance or discontinuance of the treatment in view of the composite formed by the laboratory findings and the clinical or bedside evidences. The only caution to be emphasized is the avoidance of the overestimation of relative reactions and the tendency under such circumstances to displace clinical facts by laboratory estimations. The value of the laboratory to the clinical neurologist is increasing not only as a diagnostic aid but in the prosecution of treatments. It is a branch of medicine which should be filled by trained and experienced men; and when so occupied, is entitled to the fullest measure of support and encouragement of the clinician. The nearer the cooperation of the laboratorian and the clinical neurologist, the more prompt will be the determination of many nervous and mental problems which are still obscured by the mist of our ignorance."

Bluemel[10] claims that: "One of the weak points of neuropsychiatry is the fact that diagnoses are too often rendered in terms of anatomy rather than disease, the localization of the lesion being emphasized rather than the pathologic process. As a rule such diagnoses are of little value, for the important thing is the fact that the disease is syphilitic, or tuberculous—or whatever it may be—and not that the lesion is situated a millimeter and a third to the right of the optic chiasm. When the neuropsychiatrist makes generous use of the laboratory, these regional diagnoses become less conspicuous, and clinical diagnoses come to the foreground, as they should. To establish clinical diagnoses the neurologist must demand an irreducible minimum of laboratory work. This minimum should comprise a Wassermann test and a urinalysis. The Wassermann test is the more important of the two examinations by reason of the fact that about twelve per cent of neurologic patients are luetic. Where syphilis of long standing is demonstrated or suspected, the spinal fluid should be examined as well as the blood; for the blood may be negative while the spinal fluid is strongly positive. In acute neurologic diseases, especially those with such cerebral symptoms as torpor and rigidity, the laboratory may give the exact diagnosis. Arterial blood in the spinal fluid identifies a cerebral hemorrhage; an increase in white cells in the fluid indicates an inflammatory condition and the Gram stain or the Ziehl-Neelsen stain may demonstrate the organism. Here we have a diagnosis of precision, and no such accuracy of diagnosis can be obtained by the clinician who neglects laboratory aids. In other types of neurologic cases the diagnosis is often established by such laboratory methods as blood chemistry, leucocyte counts, differential counts, etc. A cranial nerve palsy may be due to a leucemic exudate, and nothing but a white blood count will definitely identify the underlying systemic disease. In cases of coma such as fall to a neurologist's

service in a large city hospital, the diagnosis can often be rendered only through laboratory aids. The patient, being unconscious, is unable to give an account of himself, and the physical examination may not tell the full story. Here the laboratory can often solve the riddle by establishing a definite diagnosis of uremia, diabetes, cerebral hemorrhage, meningitis, etc. The clinical pathologist may render aid to the neurologist not only in diagnosis but also in the treatment of luetic cases. Even if the neurologist administers his own salvarsan and does his own spinal drainages or gives his own Swift-Ellis treatments; he will need the aid of the laboratory in checking his results, and such aid is of the highest value. The neurologist is perhaps more dependent on the laboratory than other specialists, and he can neglect the laboratory only by being a poor neurologist."

In conclusion may we quote the viewpoint of a pediatrician as expressed by Amesse,[11] who claims that: "Any open-minded survey of the progress of medicine during the past two decades would promptly disclose to surgeon and internist alike, the brilliant part played by the clinical pathologist in the development of the various medical specialties, and in their permanent establishment among the scientific bodies of the period. The advance of medicine may fairly be compared with that of the science of navigation, at first merely an art, tinctured with superstition and fantasy, resting on the uneasy basis of a false philosophy. With the dawn of civilization savage beliefs were dispelled, old wives' tales were mocked; the mariner grasped the rudiments of oceanography and learned to use his senses more and his imagination less. As the centuries went by, critical studies of the ocean currents, the tides and the winds were made, and these were exchanged among seafaring tribes, until finally, with the discovery of the compass, they could 'voyage dauntless on the sea,' and extend the domination of mankind throughout the world. The clinical laboratory is the compass of the physician. It enables him to sail fearlessly over hitherto uncharted waters; to invade wide areas closed to his predecessors, and to achieve conquests where only defeats were known. It has broadened and strengthened, sustained and stabilized the entire sphere of medicine and surgery, and has borne its full share in redeeming the tropical world from its age-old heritage of disease and death. The pediatrician acknowledges without question his dependence on the laboratory to assist with the numerous problems presented in his clinical work. For many conditions, the knowledge gained solely through this source becomes indispensable in the proper conduct of a case. For example, in diseases of the blood, differentiation, with resulting variation in prognosis and treatment, cannot be determined without the continued support of expert laboratory examinations. In the management of congenital syphilis, in the diagnosis of diphtheria, typhoid, malaria, dysentery, tuberculosis, diabetes and pyelitis, we become still further indebted. Within the past five years, investigators in research laboratories have accomplished, in preventive pediatrics alone, through the intensive study of scarlet fever, diphtheria, measles and poliomyelitis, enough to forever merit the encouragement and admiration of those dedicating their lives to the welfare of the young."

[11]Amesse, John, M.D.: Instructor Pediatrics, University of Colorado, School of Medicine.

A STUDY OF TISSUE AUTOLYSIS IN VIVO*

I. Blood Changes: Physical and Chemical

By Edward C. Mason, M.D., and Edward C. Davidson, M.D.

Assisted by C. W. Matthew and P. B. Rastello

MANN[1] and his coworkers, while developing the technic of hepatectomy, which has so greatly added to our knowledge of liver physiology, observed that a completely hepatectomized animal lived longer than an animal in which a small amount of liver substance remained free in the abdomen. The completely hepatectomized animals, when furnished sugar, lived as long as thirty-five hours, while animals having a piece of liver "the breadth of two fingers" free in the abdomen died within sixteen hours.[2] This last observation has greatly interested us since one of us has had the occasion to use a blood anticoagulant of hepatic origin ("heparin") and from its mode of preparation[3] it is reasonable to assume that the process is accompanied by marked liver autolysis. Although only 100 mg. of "heparin" was administered to each patient, such an amount frequently produced marked toxic symptoms. The degree of toxicity varying greatly with the different preparations.[4]

We were further interested to learn whether the autolysis in vivo of such an amount of liver tissue would liberate sufficient anticoagulant to be demonstrated by changes in blood coagulation. Whipple and Hurwitz[5] have reported a marked decrease in fibrinogen during experimental chloroform poisoning, while Conradi[6] has demonstrated that autolysis of various body tissues is accompanied by the production of a blood anticoagulant. Therefore, we have been interested to learn whether the reported decrease in fibrinogen was an actual decrease or only a result of autolysis such as doubtless accompanies chloroform necrosis.

After discussing these points with Doctor Mann, he has encouraged our study of these related phases of the problem.

METHOD

All operations were performed in the evening under aseptic conditions using ether anesthesia usually with a preliminary injection of morphine sulphate ¼ gr. and atropine sulphate 1/100 gr., the animals having been fed eight to ten hours prior to operation. Briefly the operation consisted of clamping the portion of the liver to be excised, ligating, sectioning, weighing and returning the sectioned liver to the abdomen. Due to the ease with which it presents, the left lobe was usually selected. However, in a few animals the small lobe lying to the left of the lobe containing the gall bladder was used. The operation usually required

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twenty to thirty minutes and the sectioned liver weighed 30 to 110 grams. In the animals where hemorrhage was thought possible a small sponge was left against the liver stump.

None of the animals showed signs of surgical shock and all were standing the following morning. The general appearance of such animals improved until within two to three hours before death, and frequently within an hour of death, their general appearance did not give warning of the impending death. Shortly before death (usually one-half to one hour) the animal lies flat on one side with all four legs extended and respiration becomes somewhat more labored. The animal approaches death without struggle except in the very terminal state when the head is drawn backwards and the chin is drawn downwards toward the neck, the terminal posture suggesting air hunger.

On postmortem examination the abdomen usually contains 100 to 300 c.c. of brown colored fluid but in no case did the fluid appear to be actual blood. A pseudofibrinous exudate is usually present, it being different from a true fibrinous exudate in that it is easily removed, leaving a smooth shining surface. A small amount of such exudate is usually present over the dome of the liver and often intestine loops are loosely adherent to each other. The intestines are generally hyperemic with marked congestion. The omentum is well wrapped about the free piece of liver; the omentum being markedly hemorrhagic and discolored. The picture is similar in many ways to that of peritonitis. However, in no case was there any indication of a septic peritonitis; and such peritonitis as is present, should doubtless be considered as chemical. Probably the most marked changes occur in the free liver tissue. It appears to have undergone a complete transformation, there being present no gross characteristics of liver tissue. The color is that of light chocolate while the feel is that of lung tissue, being spongy and gas containing. There is always a marked loss in weight of the sectioned liver ranging from six to forty grams.

A total of fifteen dogs was studied in which the following blood factors were determined: (1) Plasma Volume, (2) Serum Volume, (3) Fibrin Content, (4) Coagulation Time, (5) Amino Acids, (6) Sugar, (7) Uric Acid, (8) Urea, and (9) Nonprotein Nitrogen. For our observations, we found it best to take blood samples at the following periods: (1) at the beginning of the operation (8-9 P.M.), (2) the following morning (9-10 A.M.) and (3) before lunch. If the animal remained alive, another sample was taken about 6 P.M. and each subsequent, twelve to twenty-four hours throughout life. Of our series, two-thirds of the animals died in fifteen to eighteen hours, while two lived nearly seventy-two hours.

Plasma Volume Changes.—Plasma volume was determined through hematocrit determinations, all determinations being made in duplicate and all samples were centrifuged thirty minutes at the same rate of speed. The readings thus obtained are subject to a correction, since each ten c.c. of blood contained 1 c.c. of 1 per cent sodium oxalate solution. This source of error has been disregarded and the results have been expressed in per cent of oxalate plasma present. Chart I is presented to show the plasma volume before operation and at various periods following operation. Of the eight cases charted, it will be observed, all

show a marked decrease in plasma volume during the first twelve hours following operation. At the end of twelve hours the plasma volume either continued to decrease until the early death of the animal or soon showed an increase, and such animals lived almost seventy-two hours. While it is true two of the series showed a terminal increased plasma volume, this increase appears to be associated with the animals dying, as both samples were taken while the animals were expiring.

Serum Volume.—The lower portion of Chart I is presented to show the serum volume variations. The figures charted were obtained by allowing the blood to clot, after which the clot was broken and the tubes centrifuged as in the hematocrit determinations. The results, thus obtained, closely parallel those obtained in the plasma volume determinations, except that in some cases the

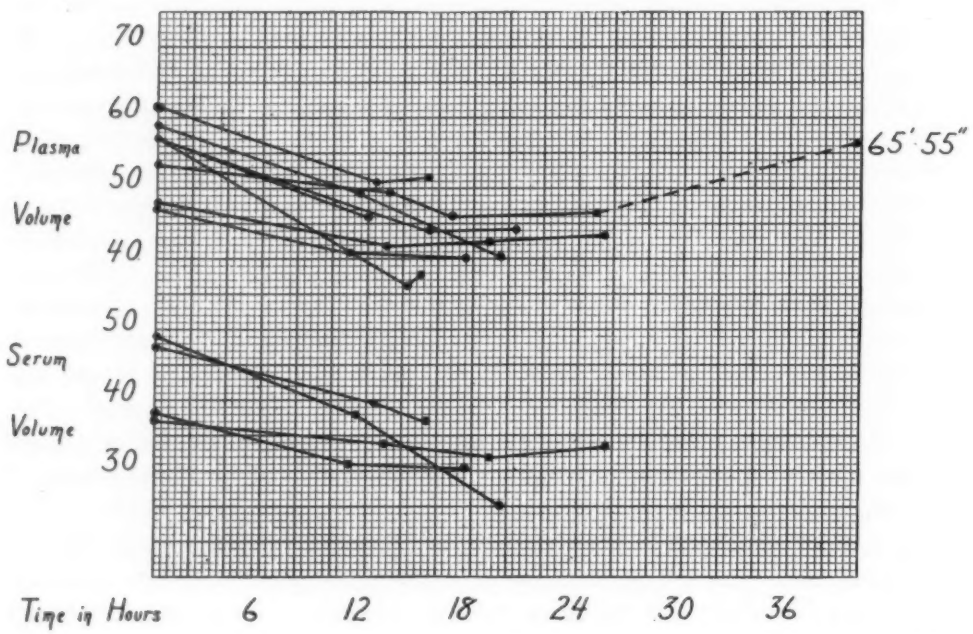

Chart I.

serum volume change is even more marked. The plasma volume in one animal changed from 59 per cent to 40.5 per cent, while the serum volume of the same animal changed from 49 per cent to twenty-five per cent. This observed change in fluid balance is apparently due to one or more of the following conditions: (1) decreased fluid intake, (2) vomiting, (3) increased hydration capacity of the tissues, (4) accumulation of fluid in the abdomen.

We did not have adequate facilities for making an accurate study of the urinary output but, from our observations, we believe that the animals do not become anuric and any lessened urinary output is doubtless associated with one or more of the above factors.

Fibrin.—Fibrin determinations were made according to the method of Foster and Whipple.[7] Briefly the method consists of recalcifying oxalate plasma, collecting the fibrin formed, drying, weighing, and igniting. The results are

briefly shown in Chart II. In all cases studied, there was a definite fibrin increase, the increase being in some cases 100 per cent. On first thought, one might consider such an increase as being due to increased blood solids accompanying the decreased plasma volume. However, comparison of the plasma volume and the fibrin content shows the concentration of fibrin continues to increase even after the plasma volume begins to return to normal (demonstrated in the charts of the two animals living seventy-two hours).

Blood Coagulations.—Coagulation determinations were made using the Howell tubes. In the series of seven cases studied, all but one showed an increase in coagulation time during the liver autolysis. The average coagulation before operating was 15.8 minutes and the average of the last samples obtained

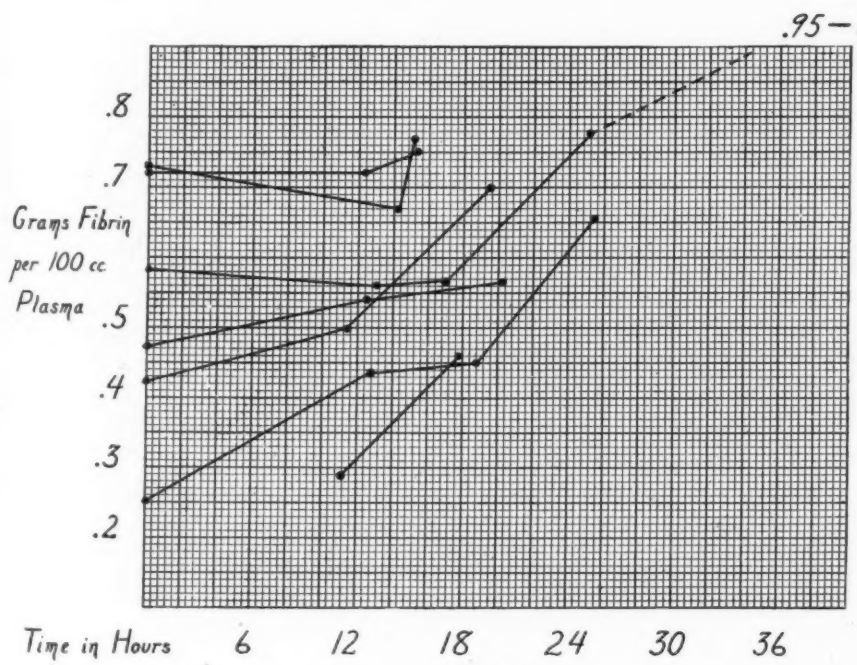

Chart II.

was 20.4 minutes. Therefore, it is suggested that autolysis of such an amount of tissue is accompanied by some delay in blood coagulation.

Amino Acids.—The Folin[8] method was used in the determination of amino acids. Chart III briefly shows the variations observed. From our findings it appears that there is a definite drop in blood amino acid nitrogen for the first period of approximately twelve hours, unless such a period ends close to the time of the animal's death. There also appears to be a very definite amino acid nitrogen increase beginning shortly before and continuing up until the animal's death. Considering the various nitrogen constituents as a possible source of toxic material which might cause the animal's death, it seems most reasonable to believe the amino acid portion most potent. It appears, however, if the toxicity is due to the amino acid fraction, that it must be a qualitative amino acid increase rather than a quantitative increase which proves fatal. This last statement is

supported by the two animals living seventy-two hours, in which case the amino acid content showed some increase above normal without death of the animal.

Sugar.—Blood sugar determinations were made according to the method of Folin and Wu; the blood filtrates being made immediately after the withdrawal of the blood and sugar determination made either immediately or the filtrate kept on ice until a satisfactory time for the determination. Chart IV shows the usual type of sugar curve obtained. However, in one animal the sugar was higher at the end of twelve hours than at the onset of the experiment; and in another, it was higher at death than at the beginning of the experiment. We are rather inclined to believe these two results may not be reliable and that the curve presented in Chart IV represents what actually happens in blood sugar

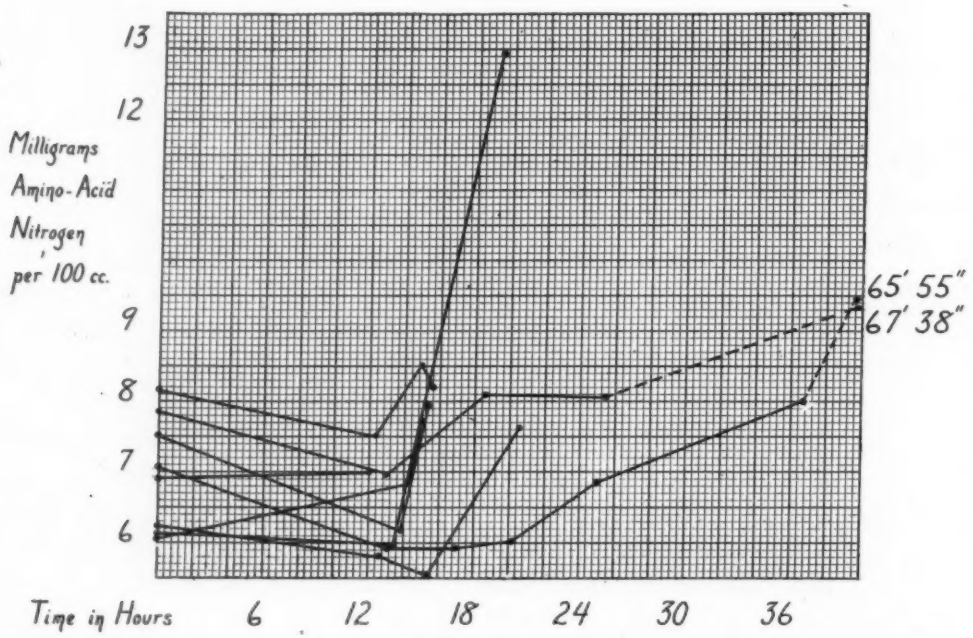

Chart III.

concentration. There appears to be a pronounced drop in blood sugar during the first twelve hours, following which the continued drop is more gradual until the early death of the animal. The two animals of our series which lived nearly seventy-two hours showed a gradual increase in blood sugar following the first period of eighteen to twenty-four hours. While the blood sugar drop is quite marked, it is not sufficient to account for death of the animals. The lowest level was 53 mg. per 100 c.c. blood.

Urea Nitrogen.—Urea determinations were made according to the method of Van Slyke and Cullen. The results of such determinations are illustrated by Chart V, the urea concentration becoming less during the first twelve hours and rising rather abruptly before death. The curve is very similar to that of the amino acids and, therefore, also to the nonprotein nitrogen curve. Although the urea nitrogen curve is similar to the amino acid curve, it apparently

is not as good an index of the animals condition; for it will be noted that the amino acid fraction often shows a rise, while the urea fraction is still low.

Uric Acid.—Morgulis and Edwards[9] found uric acid to be 0.9 to 2.2 mg. per 100 c.c. in the normal dog. Hayden and Orr[10] found the amount of uric

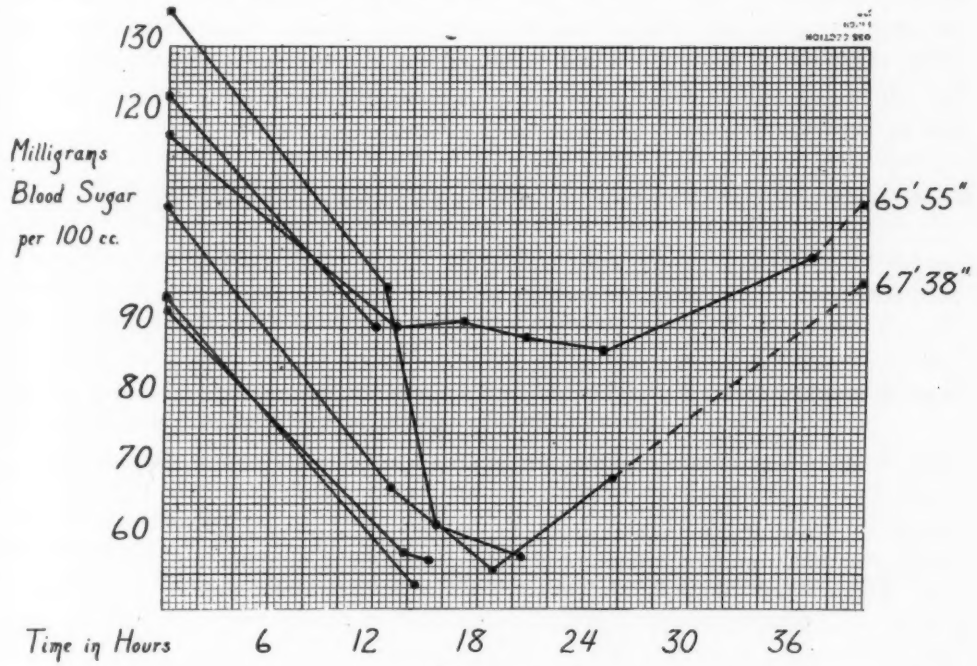

Chart IV.

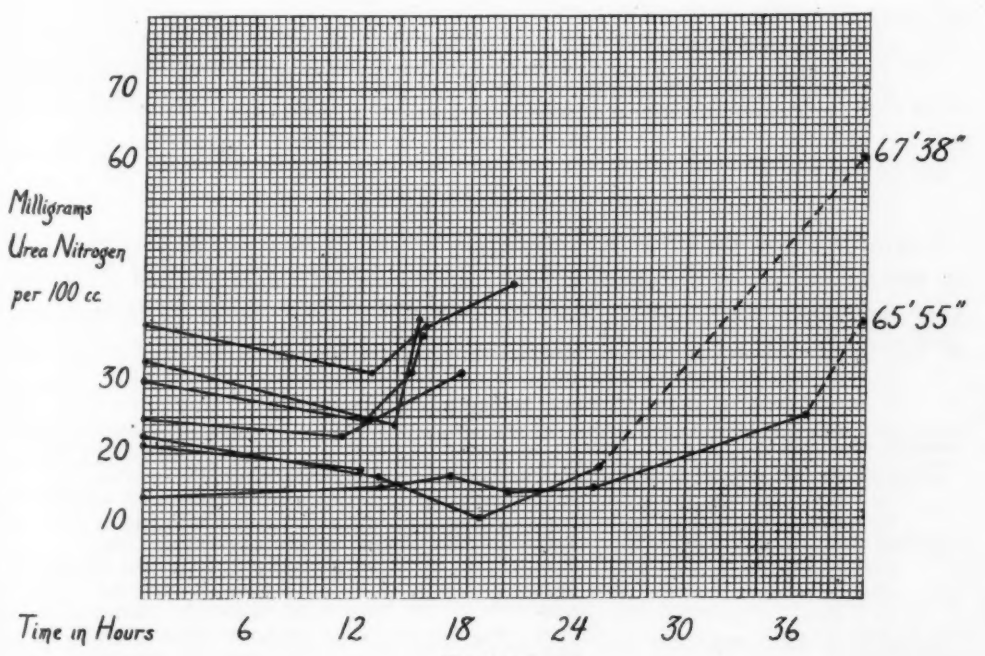

Chart V.

acid to be 1.5 mg. per 100 c.c. and Draper[11] gives a figure of 0.7 mg. per 100 c.c. We found the uric acid to be nil to 2 mg. per 100 c.c. in the normal dog. The uric acid showed a definite increase in three-fourths of our animals, the increase ranging from 0.5 mg. to 3.7 mg. per 100 c.c., the sample from the latter animal being taken while the animal was dying. The uric acid increase was not usually noted until the animal was very near death. Chart VI is a composite chart of all factors studied in an animal living approximately seventy-two hours. It will be noted that the uric acid increase did not appear until late in the experiment.

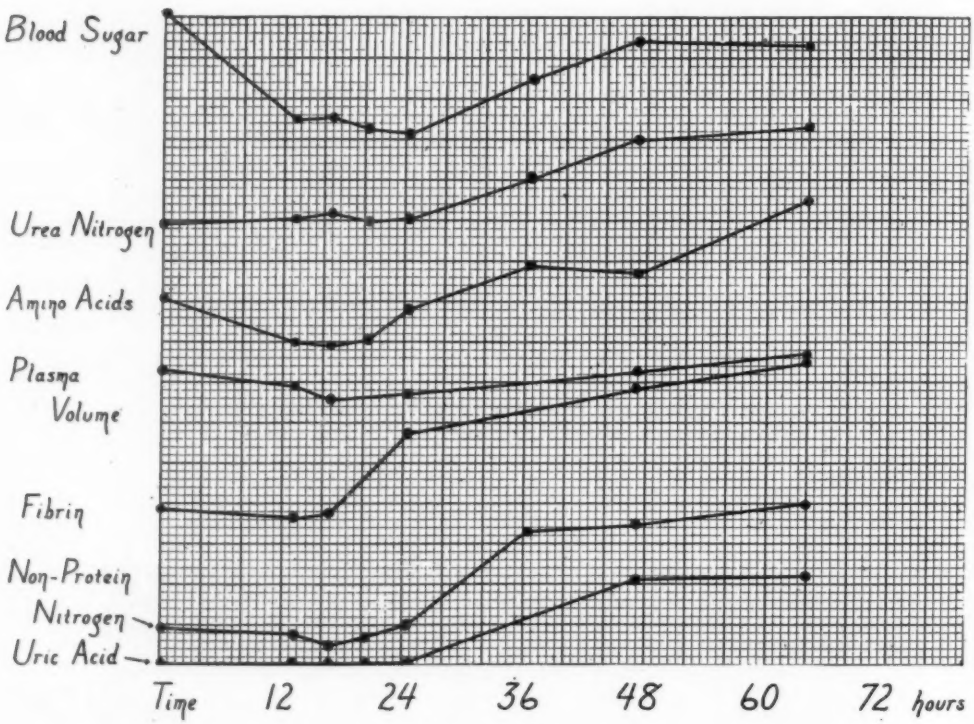

Chart VI.

The Relation Between the Proportion of the Liver Sectioned to the Size of the Animal and the Period of Life Expectancy.—It is reasonable to expect that the larger the sectioned piece of liver the greater would be the amount of toxic material liberated and consequently the earlier the death of the animal.

TABLE I

WEIGHT OF ANIMAL	WEIGHT OF LIVER	PERIOD OF LIFE FOLLOWING
20.4 K	43.5 grams	20 hours 15 minutes
10.4 K	57.5 "	Alive 67 hours 38 minutes
10.9 K	110.0 "	15 hours 45 minutes
13.6 K	39.0 "	20 hours 24 minutes
13.1 K	32.0 "	Alive 65 hours 55 minutes
13.1 K	40.0 "	15 hours 21 minutes
11.3 K	42.0 "	15 hours 15 minutes
19.3 K	78.0 "	16 hours 30 minutes
18.0 K	86.0 "	17 hours 57 minutes

Our experiments have definitely shown that such reasoning does not hold. Table I briefly summarizes our findings on this point, the facts recorded being the weight of the dog in kilos, the weight of the sectioned liver left in the abdomen and the period of life following.

The length of time an animal will live following sectioning of a piece of liver depends upon: (1) the rate of liberation of the toxic substance, and (2) the rate of absorption of such substance.

DISCUSSION

The adsorption of all dead or injured body tissue, as well as organic foreign material introduced into the body, is accomplished principally through the action of autolysis. The degree of toxicity accompanying such adsorption apparently depends upon the toxicity of the by-products liberated during autolysis. With this conception of autolysis, it becomes necessary to assume that the toxicity accompanying burns is possibly due to the rapid liberation of such toxic by-product and it is likewise highly suggestive that the malaise accompanying x-ray radiation is the result of the liberation of a toxic material associated with autolysis of body tissue.

It is interesting that several of the blood constituents of our animals vary in a manner somewhat similar to those reported in completely hepatectomized animals by Mann and his coworkers. We have observed a decrease in blood urea for the first twelve hours which Bollman, Mann and Magath have also observed. However, they report a continued decrease until death while our animals showed a terminal increase. We have also noted a marked decrease in blood sugar but again the decrease is not as great as that observed in the completely hepatectomized animals. Our findings in uric acid variations have been quite similar to those reported on the completely hepatectomized animals. Yet, we do not wish to imply that the physiology of the animals we have studied is comparable with that of the completely hepatectomized animals.

CONCLUSIONS

1. Autolysis of liver tissue free within the abdominal cavity is accompanied by the production of a highly toxic substance.

2. The length of life following sectioning of the liver does not depend upon the proportion of liver sectioned to the weight of the animal.

3. Accompanying the autolysis of the sectioned liver there appears:
   (a) Decreased plasma volume.
   (b) Decreased serum volume.
   (c) Slightly prolonged coagulation time.
   (d) Increased fibrin content.
   (e) Decreased amino acid nitrogen during the first period of approximately twelve hours, followed by a terminal increase.
   (f) Decreased blood sugar until death except in the two animals living seventy-two hours.
   (g) Increased uric acid in two-thirds of the cases.
   (h) Decreased urea nitrogen and nonprotein nitrogen with a terminal increase.

4. Blood studies show marked and constant changes. However, such changes do not account for the toxic element causing the death of such animals.

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II. THE POINTS OF ACTION OF SODIUM PHENOBARBITAL AND PHENOBARBITAL IN LOWERING BLOOD PRESSURE*

By Charles M. Gruber, Ph.D., M.D., and Roy F. Baskett, M.D., St. Louis

IN the twelve years since the introduction of luminal and luminal sodium a great deal of work has been done on their uses in the treatment of epilepsy and as hypnotics. Even with the vast number of such cases treated and the consequent wealth of material to work upon no one seems to have noted how or where these effects are accomplished. The literature on the subject yields only three investigators who mention their possible effect on blood pressure.

In 1912, Impens[1] noted that luminal and luminal sodium were able to lower the blood pressure in experimental animals, and in some cases produced an additional rise. Wetzel,[2] after failure to find any effect upon the circulation when these drugs were given to experimental animals in hypnotic doses, made note of the fact. Gregor[3] observed that luminal lowers the blood pressure in human beings.

Recently we have shown[4] that sodium phenobarbital and phenobarbital increased the blood pressure twice in over one hundred and sixty-four injec-

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tions in thirty-two dogs and nine rabbits. This subsequent investigation was undertaken to determine the effect of these drugs upon 1, the heart; 2, the volume of the limb, kidney, intestine and spleen; and 3, to note whether the change in the volume of the organs is due to the central or the peripheral action of these drugs, in other words, the point of action.

METHOD

Dogs, cats and rabbits were used. The dogs were anesthetized with ether by inhalation or with paraldehyde (1.8 ml. per kilo per os). The cats and rabbits were anesthetized with urethane 2 gm. per kilo body weight by stomach. After anesthetization the animals were tracheotomized.

In all the experiments, except those in which perfusion was employed, the blood pressure was taken from a cannula inserted in the left carotid artery and connected with a mercury manometer. A two per cent sodium

Fig. 1.—Cat, 3 kilograms, urethane anesthesia. Artificial respiration. Heart plethysmographed. *2*, Record of heart; *3*, blood pressure; *4*, time in fifteen seconds and zero blood pressure and *5*, time and duration of injection of drug. *1*, 0.2 gm. sodium phenobarbital dissolved in 2 ml. Ringer's solution injected into the external jugular vein. Up stroke diastole, down stroke of lever systole.

citrate solution was used as an anticoagulant. A chronograph marking either five, fifteen or thirty second intervals was placed at the zero blood pressure level. The writing points were placed one above the other.

The organs of the abdomen were exposed through a midline incision. For plethysmographing a loop of intestine, a kidney or the spleen, metal oncometers were used connected with piston recorders which marked the changes in volume upon the drum surface. These were applied with as little manipulation of the organs as possible and the abdomen then sewed up. A metal leg oncometer connected to a piston recorder was used in recording changes in the volume of the hind limb. In all instances before the oncometer was applied the limb was disjointed at the ankle, so that the foot was not used in the plethysmograph oncometer. The limb and at least one other organ were always plethysmographed simultaneously and in many cases

the volume changes of three organs were recorded at once in addition to the recording of blood pressure.

After establishing artificial respiration the changes in heart volume were recorded by placing a glass cardiometer about the heart, exposed by excising the ventral half of the thorax with the loss of as little blood as possible. Changes in blood pressure were recorded simultaneously. In a number of instances records of both auricular and ventricular contractions were made simultaneously with cardiographs[5] applied directly to the heart.

In a number of cats the kidneys, loops of intestine and hind limbs were perfused. The perfusion method was essentially the same as that used by Sherrington and Sowton.[6] The free end of the arterial cannula was connected to a short rubber tube leading from a reservoir containing a Ringer's solution, modified by adding defibrinated blood from the animal. It was kept in a water-bath at 38.5° C. and oxygenated by passing air through it. The perfusion pressure varied from 60 mm. of mercury to 100 mm. but was kept practically uniform throughout each experiment, maintained by the rate of flow of air bubbling through the perfusion fluid. The excess air was allowed to escape through a mercury valve which supported the desired pressure. During the escape of air through the valve the pressure would fall 4 mm. of mercury. This variation occurred about every five to ten seconds. Sodium phenobarbital dissolved in the perfusion mixture 0.1 gm. per ml. was injected into the perfusate near the arterial cannula. Such injections had no effect upon the perfusion pressure as this was equalized by the escape of air through the mercury valve.

The drops of perfusion fluid, flowing from a glass cannula placed in the vein leading from the organ, were recorded on the kymograph by means of a receiving and a recording tambour.

The effect upon urine secretion was noted in a number of cases. The flow was recorded by placing metal cannulas into the ureters. Sodium phenobarbital* was always injected, except as previously stated, dissolved in Ringer's solution, 0.1 gram per ml. Phenobarbital* in the few instances studied was dissolved in ethyl alcohol 0.1 gm. per ml. alcohol.

In the cats and dogs these injections were made directly into the exposed femoral vein. In the rabbits the drugs were injected into the lateral ear vein.

RESULTS

The Effect upon the Intact Heart Muscle.—If the blood pressure records are studied closely it will be noted that the heart rate was slowed in all records in which large doses were used. The average slowing of heart rate in dogs by 0.1 gm. sodium phenobarbital was 6.1 per cent; in the rabbits the same dose caused a slowing in heart rate of 8.1 per cent. A slowing equivalent to that produced in dogs by 0.1 gm. was produced in rabbits by 50 mg. of sodium phenobarbital. In the dogs 0.2 gm. sodium phenobarbital decreased the rate 13.3 per cent, and 0.4 gm. caused an average slowing of 28.8 per cent. As this effect was not influenced by the cutting of the vagi,

*The preparations used were presented to us by the Winthrop Chemical Company.

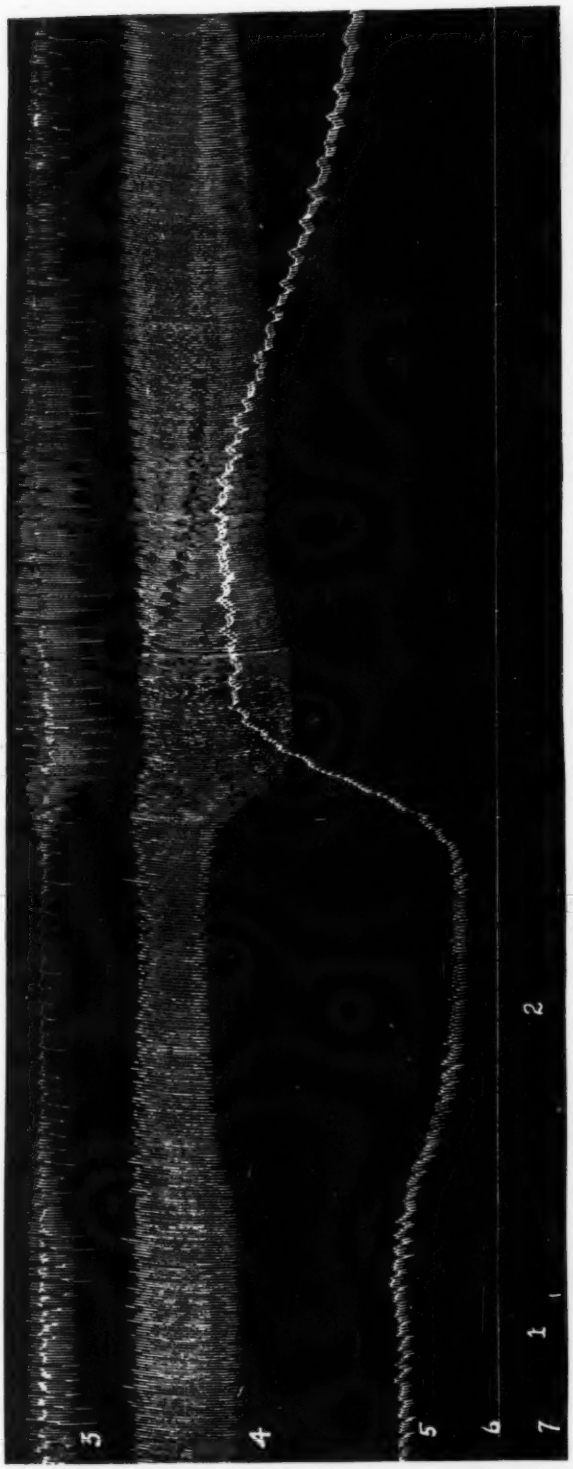

Fig. 2.—Dog, 14 kilograms, paraldehyde anesthesia. Artificial respiration. Up stroke of heart lever diastole, down stroke systole. 3, auricles; 4, ventricles; 5, blood pressure; 6, time in fifteen seconds and zero blood pressure and 7, time and duration of injections. 1, 0.4 gm. sodium phenobarbital dissolved in 4 ml. of Ringer's solution; 2, 1 ml. adrenalin chloride 1:10,000 solution.

the question therefore arose as to what effect sodium phenobarbital and phenobarbital may have upon the heart muscle itself.

The hearts of both cats and dogs were plethysmographed. It was noted that small doses, 25 mg. in cats and 50 mg. in dogs, had no effect upon the heart volume. Fifty mg. in cats and 0.1 gm. in dogs, when injected intravenously, caused an increase in the volume of the heart, i.e., the heart dilated. Larger doses produced further dilation but in our experiments never caused cessation of heart beat. Fig. 1 is presented as typical of all such experiments. A cat weighing three kilograms was anesthetized with urethane. The thorax was opened and the heart, exposed under artificial respiration with as little loss of blood as possible, was placed in a glass oncometer and the contractions recorded. The down stroke shows the systolic and the up stroke the diastolic contraction, any change in the curve upward indicates increased volume of the heart. At 1, 0.2 gm. sodium phenobarbital was injected into the external jugular vein. There resulted immediately a fall in blood pressure accompanied by an increase in the volume of the heart. Similar changes were noted in both cats and dogs.

The effect of these drugs upon the contractile power of both the auricles and ventricles was also studied. Small doses had no effect upon either the auricles or ventricles; larger doses caused practically complete cessation of contraction of the auricles and marked decrease in the length of the excursions of the ventricles and also a decreased rate of beat. (See Fig. 2.) In Fig. 2, a dog weighing 14 kilograms was anesthetized with paraldehyde and cardiographs were attached to both auricles and ventricles. At 1, 0.4 gm. sodium phenobarbital dissolved in 4 ml. Ringer's solution was injected into the femoral vein. Shortly thereafter the auricles ceased beating, the ventricles continuing to beat but at a much slower rate and with decreased force. Sodium phenobarbital, like most drugs, was found to be more toxic to the auricles than to the ventricles. Upon hearts so depressed we tried the effects of various so-called cardiac stimulants. Large doses of camphor, 20 ml. of a saturated solution, in Ringer's were rapidly injected intravenously without effect. Likewise 8 mg. strychnine sulphate were injected intravenously without effect. Adrenalin chloride on the other hand injected intravenously always markedly increased the force and rate of contraction. In Fig. 2 at 2, 1 ml. of a 1:10,000 solution of adrenalin was injected intravenously after section of both vagi. An immediate escape of the heart from the action of sodium phenobarbital was noted. The reverse condition will be seen in Fig. 3, in which the same animal was used, the adrenalin chloride being injected before the sodium phenobarbital. In this instance 1 ml. of a 1:10,000 adrenalin chloride solution was injected at 1 and 0.4 gm. sodium phenobarbital dissolved in 4 ml. Ringer's solution was injected at 2. The sodium phenobarbital even in the presence of adrenalin was able to slow the rate and decrease the force of the heart beat. Similar results were obtained with phenobarbital. It must be admitted that a part at least of the fall in blood pressure may be due to the effect of sodium phenobarbital and phenobarbital upon the heart muscle itself.

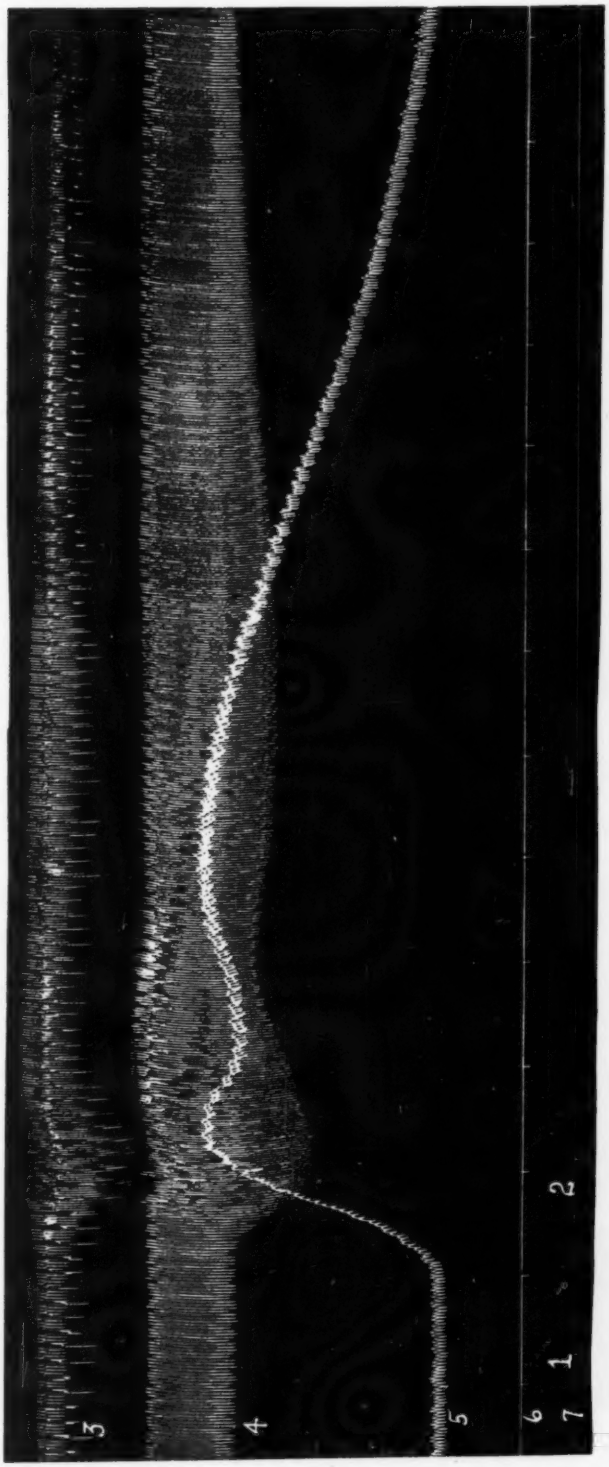

FIG. 3.—Same as Fig. 2, except at *1*, 1 ml. adrenalin chloride 1:10,000 was injected and at *2*, 0.4 gm. sodium phenobarbital was administered.

The Effect of Sodium Phenobarbital upon the Volume of the Limb, Kidney, Spleen and Intestine.—In order to determine whether or not the fall in blood pressure brought about by sodium phenobarbital injection is the result of a weakened myocardium only, or whether the vascular system is also involved, the limb, kidney, spleen and intestine were plethysmographed to see if any

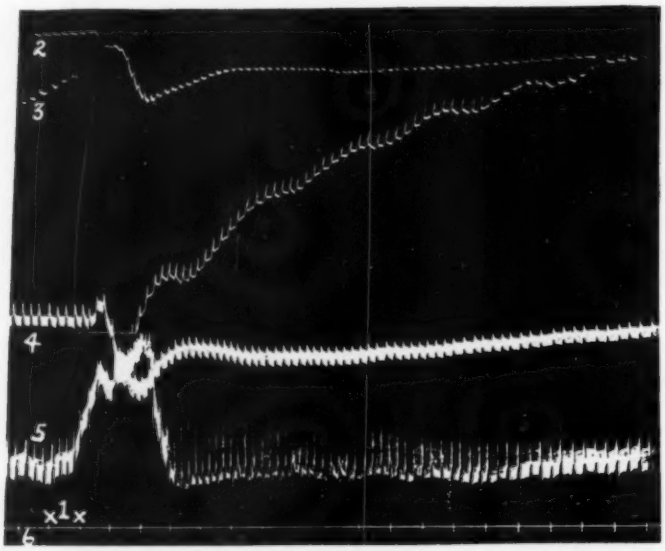

Fig. 4.—Dog, 12 kilograms, paraldehyde anesthesia, artificial respiration. *2*, Record of volume of kidney; *3*, volume of spleen, and *5*, volume of limb. *4*, Blood pressure record; *6*, time in fifteen second intervals and zero blood pressure. *1*, 0.2 gm. sodium phenobarbital injected intravenously. The blood pressure wrote 8 mm. in advance of all plethysmograph writing points. Up stroke of plethysmograph record indicates increased volume of organ, down stroke decreased volume of organ.

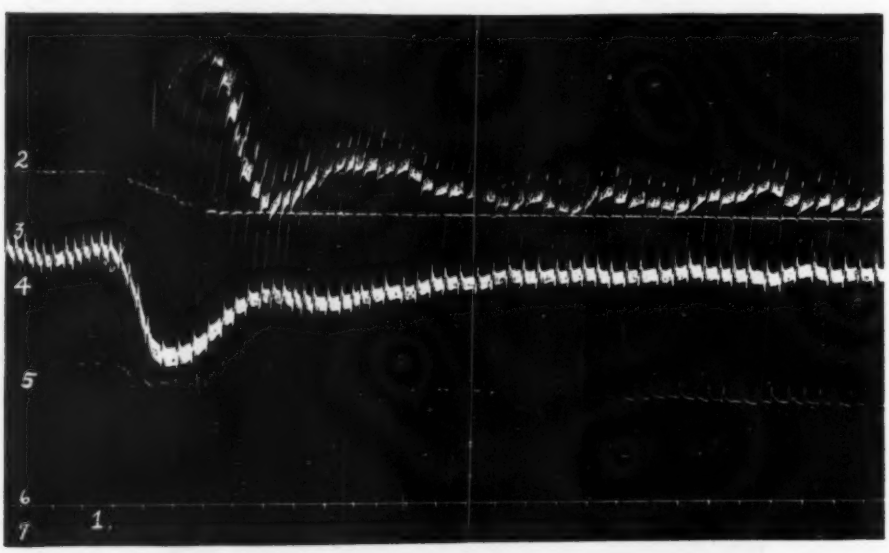

Fig. 5.—Dog, 16.4 kilograms, paraldehyde anesthesia. *2*, Record of volume of kidney; *3*, blood pressure; *4*, volume of limb; *5*, volume of intestine; *6*, time in fifteen second intervals and zero blood pressure, and *7*, point and duration of injection of drug. *1*, 0.2 gm. sodium phenobarbital injected intravenously. Up stroke increase in volume, down stroke decreased volume of organs.

change in volume occurred during the fall in blood pressure. In the animals so studied with two exceptions, sodium phenobarbital produced an increase in limb volume and a decreased volume of the spleen, of the kidney and usually of the intestine. In two animals the kidney increased in volume and the limb decreased with all the injections. Figs. 4 and 5 show the usual results. In Fig. 4, the blood pressure and the volumes of the kidney, spleen and limb were recorded. It will be noted that the kidney and spleen markedly diminished in volume while the limb increased. A similar condition is seen in Fig. 5. In this case the kidney and intestine decreased in volume simultaneously with a fall in the blood pressure and marked dilation of the limb. The dog in Fig. 4, weighed 12 kilograms and was under artificial respiration; the one used in Fig. 5, weighed 16.4 kilograms. Both animals received 0.2 gm. sodium phenobarbital dissolved in Ringer's solution intravenously at 1.

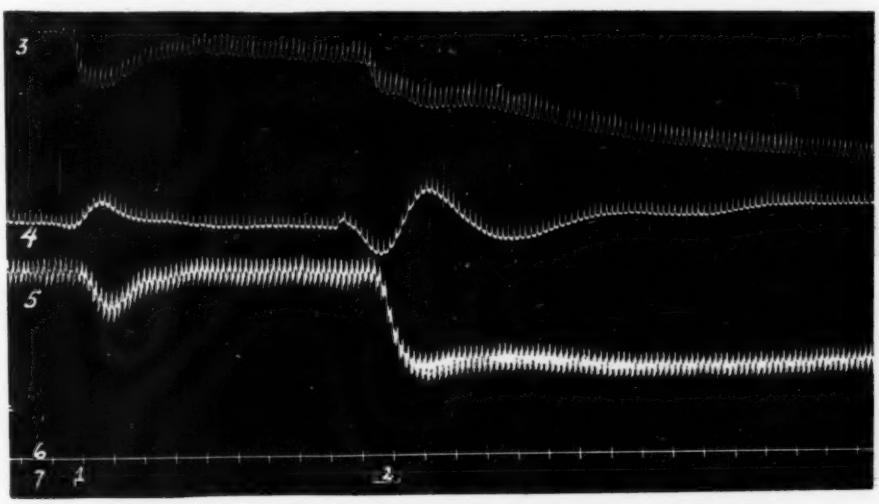

Fig. 6.—Same as Fig. 4. *3*, volume of kidney; *4*, volume of limb; *5*, blood pressure; *6*, time interval in fifteen seconds and zero blood pressure, and *7*, point and duration of injection. *1*, 2 ml. ethyl alcohol injected intravenously. *2*, 2 ml. ethyl alcohol in which has been dissolved 0.2 gm. phenobarbital injected intravenously.

Fig. 6 is presented to show the effect of phenobarbital upon the volumes of the kidney and limb. The results are similar to those obtained with sodium phenobarbital. The same animal from which Fig. 4 was made was used, artificial respiration being continued throughout the experiment. At 1, 2 ml. ethyl alcohol were injected intravenously and at 2 an equal amount of alcohol containing 0.2 gm. phenobarbital. In 2, a temporary decrease in limb volume is noted, due to the sudden fall in blood pressure (passive constriction) but this is quickly compensated for, by an active increase in volume. The kidney decreased in volume after each injection. Both kidney and limb volumes returned to normal later, which fact does not appear in the record. Active dilation of the vessels of the limb seems to result from administration of sodium phenobarbital and phenobarbital.

The Effect of Sodium Phenobarbital Injected into the Perfusate upon the Vessels of the Excised Limb, Kidney and Intestine.—In order to determine if sodium phenobarbital has a peripheral action upon the vessel wall, experiments were performed in which the limb, kidney and loops of intestine were perfused with Ringer's solution containing defibrinated blood. The sodium phenobarbital was dissolved in the modified Ringer's solution and was injected into the perfusate through the rubber tubing near the arterial cannula. Figs. 7, 8 and 9 are records showing the effect of such injections upon the rate of flow of the perfusion fluid from the limb, kidney and intestine respectively. In Fig. 7, the gracilis, adductor femoris and semimembranosus muscles of the cat were perfused through the ramus muscularis branch of the femoralis artery[7] at a pressure of 60 mm. of mercury. At 1, 0.2 gm. sodium phenobarbital dissolved in 2 ml. blood Ringer's solution was injected into the perfusate. The rate of flow increased suddenly from 22 to a maximum of 58 drops per thirty seconds or an increase of 164 per cent. These results were unvariable. The blood vessels of the limbs of two animals simi-

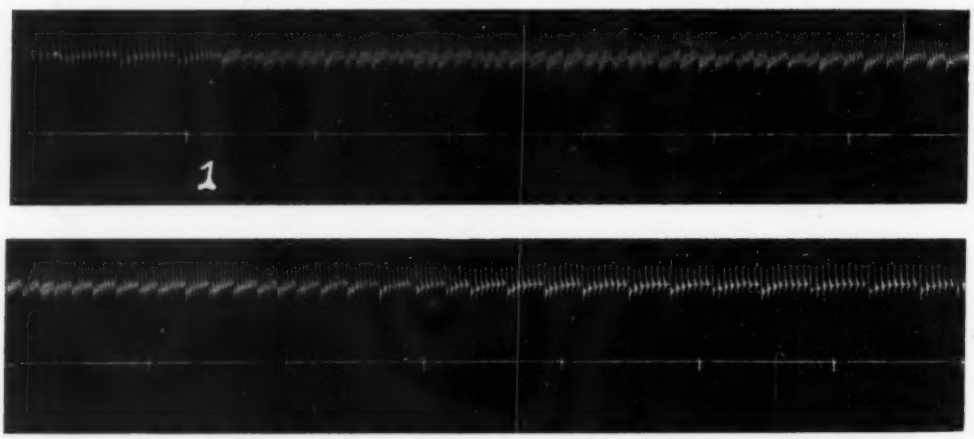

Fig. 7.—Muscles of a cat perfused with 38.5° C. Ringer's solution containing defibrinated blood. Perfusion pressure 60 mm. of mercury. Top record rate of flow of fluid from the muscles in drops. Middle record time in thirty second intervals. Bottom record, at *1*, 0.2 gm. sodium phenobarbital dissolved in 2 ml. of the perfusion fluid was injected into the perfusate.

larly treated, responded by a decreased flow of the perfusion fluid. The effect upon the kidney vessels differed from that upon the vessels of the limb. In Fig. 8, a cat's kidney was perfused at 90 mm. of mercury pressure. At 1, 0.2 gm. sodium phenobarbital was injected into the perfusate. There was an immediate increase in rate of perfusion flow, from 26 to 32 drops per thirty seconds which quickly decreased to ten drops for the same period. This vasoconstriction was followed later by a prolonged vasodilation in which the maximum rate of flow was 50 drops per thirty seconds, an increase of almost one hundred per cent. This change in blood flow with large doses was quite uniform throughout the experiments. The first increase was not due to increased pressure resulting from the injection but was due to the effect of the drug itself.

The results from perfusing the loop of intestine were uncertain. Usually

a decreased rate was observed. Fig. 9 is one in which a definite constriction was obtained. One-fifth gm. sodium phenobarbital dissolved in 2 ml. solution was injected into the perfusate. The rate of flow immediately decreased from 70 to 30 drops per thirty seconds or a decrease of 57 per cent.

The Effect of Sodium Phenobarbital upon the Secretion of Urine.—Although phenobarbital has an effect upon the kidneys, in the few animals tested it appeared to have no direct action on urine secretion. Fig. 10, is a record from a 14 kilogram dog in which sodium phenobarbital 0.3 gm. was injected at 1. There was a temporary cessation in the secretion due, we believe, to the fall in blood pressure and not to a direct effect upon the kidney. How-

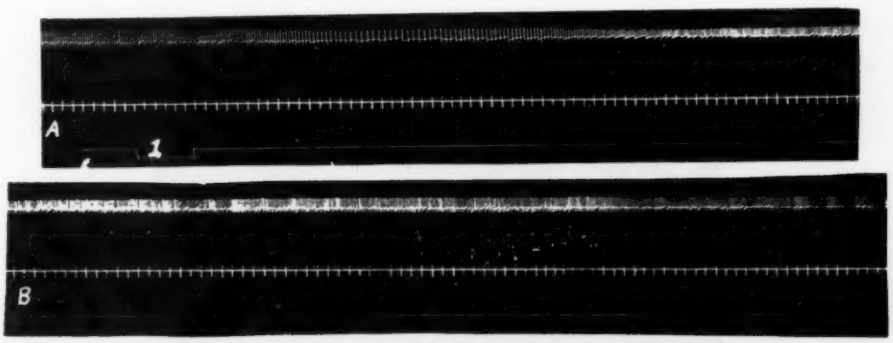

Fig. 8.—Cat's kidney perfused with 38.5° C. Ringer's solution containing blood. Perfusion pressure was 90 mm. of mercury. Top record flow of fluid from the kidney in drops; middle record time in five second intervals and bottom record, at *1*, 0.2 gm. sodium phenobarbital was injected into the perfusate. *B*, continuation of *A*.

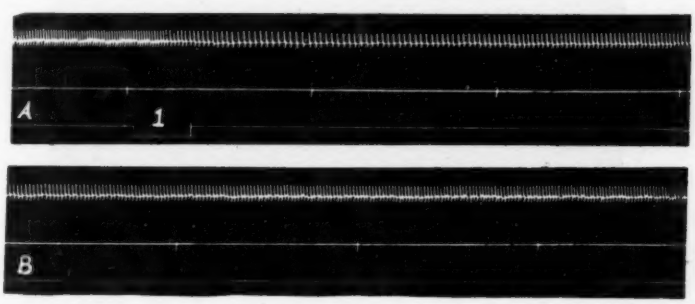

Fig. 9.—Loop of cat's intestine perfused with Ringer's solution containing blood. Perfusion pressure was 90 mm. of mercury and temperature of the fluid 38.5° C. Top record flow of fluid from loop of intestine in drops, middle record time in thirty seconds, bottom record at *1*, 0.2 gm. sodium phenobarbital was injected into the perfusion system. *B*, continuation of *A*.

ever, in those cases in which the blood pressure remained permanently low after repeated small injections or after the injection of a large dose, the secretion of urine was usually diminished and in some cases anuria resulted. This again we believe to be due to the effect of sodium phenobarbital upon the blood pressure and not upon the kidney. Although sodium phenobarbital in large doses as in perfusion experiments is capable of causing vasoconstriction of the vessels of the kidney, it does not seem possible that when given by mouth it can be sufficiently concentrated to interfere with the urine secretion.

DISCUSSION

Many of the earlier writers believed that the heart rate was accelerated under the influence of phenobarbital and sodium phenobarbital. With our results on experimental animals we have been unable to confirm this. We found as the usual effect that the heart rate is slowed, the heart's force is diminished and that the heart itself is dilated slightly when acted upon by sodium phenobarbital. There appears to be a greater depression of the cutaneous vessels than of the visceral vessels and that the vessels of the limb dilate while those of the intact intestine, kidney and spleen constrict. However, the perfusion experiments show that the vessels of the kidneys may also dilate and those of the limb may constrict.

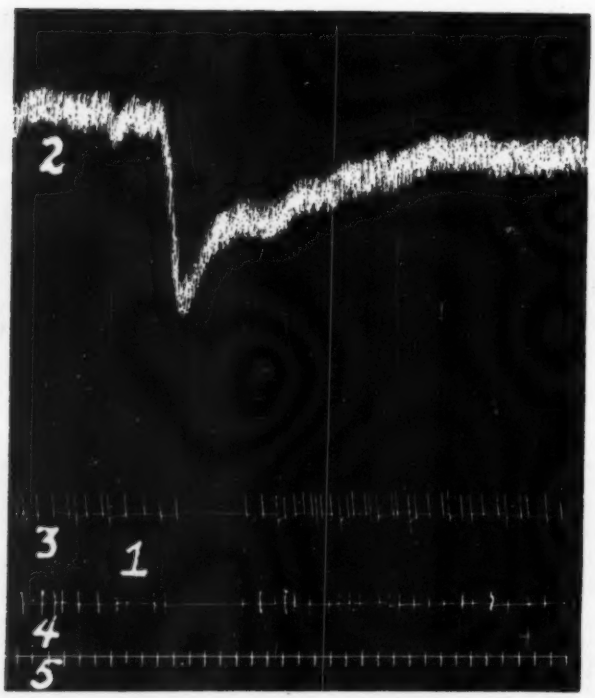

Fig. 10.—Dog, 14 kilograms, paraldehyde anesthesia. Top record blood pressure with mercury manometer, bottom record *5*, time in fifteen second intervals and zero blood pressure. Records *3* and *4* are the rate of flow of urine from the left and right kidneys respectively. *1*, 0.3 gm. sodium phenobarbital injected intravenously.

SUMMARY

1. Phenobarbital and sodium phenobarbital, when injected intravenously, cause slowing of the heart rate and decrease of the force of the heart beat.

2. Phenobarbital and sodium phenobarbital, injected intravenously cause slight dilation of the heart..

3. Phenobarbital and sodium phenobarbital, given intravenously usually decrease the volume of the spleen, kidney and loop of intestine, but cause an increase in volume of the limb.

4. Perfusion experiments of the kidney, limb and loop of intestine show the action of sodium phenobarbital to be peripheral and not central in origin.

5. Perfusion experiments on the kidney and limb show that sodium phenobarbital may cause dilation of the vessels in these organs as well as constriction.

6. Sodium phenobarbital in moderate doses has no direct action upon the secretion of urine, although if given in large doses the resulting fall in blood pressure is capable of decreasing the urine secretion even to the point of causing anuria.

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SYNERGISTIC ANALGESIA AND ANESTHESIA WITH SPECIAL REFERENCE TO MAGNESIUM SULPHATE, ETHER, MORPHINE AND NOVOCAINE*

By James T. Gwathmey, M.D., and Charles W. Hooper, M.D., New York City

THE late Samuel J. Meltzer entertained and defended the view that the phenomena of life are not simply the outcome of a single factor of excitation, but that they are the results of a compromise between two antagonistic factors, the fundamental forces of life—excitation and inhibition. In a series of experiments on the effects of intracerebral injections of solutions of various salts carried out in 1898, Meltzer observed that an intracerebral injection of two drops of a 5 per cent solution of magnesium sulphate produced complete anesthesia and relaxation of the animal lasting several hours. On the basis of the hypothesis that magnesium is an inhibitory factor in the life phenomena, this observation led to a prolonged experimental study of the effects of magnesium salts on the animal body and to many publications on this subject. In their first publication in 1905, Meltzer and Auer made the general statement that the salts of magnesium are capable of inhibiting the entire nervous system, and assumed in particular that these salts are also capable of producing anesthesia and other inhibitory effects on the central nervous system. They found that after an injection of a proper dose of a solution of magnesium sulphate the animal loses for some time all reflexes and signs of sensibility, while the respiration remains intact. This first publication led to an extensive literature on the subject. Some of the writers insisted that magnesium exerts only a curare-like action, that is, the salts

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paralyze the motor nerve endings to such a degree that the animal is incapable of responding to any stimulation; in other words, that the animal which appears to be anesthetized is actually conscious during the operation and feels all the pain inflicted on it, but is incapable of manifesting its sensations on account of the complete paralysis. Meltzer and his coworkers labored for many years to establish magnesium sulphate as an anesthetic agent. They investigated the effect of magnesium salts on animals and in a comparatively considerable degree also on human beings by different modes of administration: by intravenous, intraspinal, intramuscular and subcutaneous injections. In all modes of administration the effect was found to be unmistakably depressing in character. They found among other things that the hypodermic injection of a concentrated solution of magnesium sulphate had a profound depressing effect upon the nervous system without any preceding excitation. The subcutaneous injection of magnesium sulphate never led to an immediate or late appearance of diarrhea or more frequent stools. The salt is eliminated to a great extent through the kidneys. They further observed that calcium antagonizes the abnormal activity of its three inorganic associates in the animal body, sodium, potassium and magnesium, be the activity an over-inhibition of an over-excitation.

In 1916, Peck and Meltzer employed magnesium sulphate clinically as an anesthetic in man. These observations proved conclusively that the state of anesthesia which is produced by injection of magnesium sulphate is actually anesthesia, that is, in this state sensation as well as consciousness are temporarily abolished. They found that the anesthesia may or may not be accompanied by a pronounced paralysis of the endings of the motor nerves of a great part of all skeletal muscles. The central effect, especially the effect on the sensation of pain and on consciousness, can be attained with a smaller dose of magnesium sulphate than that which is required for a paralysis of the motor nerve endings. The central effect also appeared to set in sooner than the peripheral one.

I. SYNERGISTIC ACTION OF MAGNESIUM SULPHATE AND ETHER

Meltzer and Auer, 1913-14, made the surprising observations that after the intramuscular injection of one-half or less of the anesthetic dose of magnesium sulphate, rabbits and dogs are readily and deeply anesthetized by inhalations of small doses of ether which are insufficient to anesthetize normal animals. They found the anesthetic dose of magnesium sulphate by intramuscular injection to be about 1.2 grams per kilo for the rabbit and between 1.75 and 2 grams per kilo for the dog. In the experiments which they reported, rabbits were given 0.6 gram (or less) and dogs 0.8 gram (or less) per kilo; with these small doses the animals were only slightly affected; if ether was insufflated eight or ten minutes after such an injection, the animals became deeply anesthetized. The dose of ether which was sufficient to cause deep anesthesia was hardly a tenth of the dose which was otherwise required for a similar deep anesthesia without the help of magnesium sulphate. Each experiment consisted of a series of three animals; for example, one dog

received 0.8 gram magnesium sulphate per kilo, another inhaled a small dose of ether, and the third inhaled an equal amount of ether after it had been injected with 0.8 gram magnesium sulphate per kilo. The ether was administered simultaneously to the second and third animals by means of cannulae of equal size from the same ether flask. The first and second animals were only slightly affected. The third animal always became deeply anesthetized, and the course of the anesthesia was as follows: if a stream of air containing about 3 per cent ether was administered, deep anesthesia developed after a few minutes, in which the abdomen or pleural cavity could be opened, the parietal peritoneum or parietal pleura scratched and the sciatic nerve exposed, taken up on a thread and torn, without a sign of pain sensation or other reaction. In the first stage the lid reflex remained quite prompt and sometimes short, apparently spontaneous winking motions occurred; irritation of the central end of the vagus caused cessation of breathing which was proceeding regularly; irritation of the sciatic gave a strong normal contraction of its muscle group. Soon every reaction upon irritation of the sciatic disappeared. *By the administration of smaller doses of magnesium sulphate and ether the first stage anesthesia was reached and maintained, that is, complete lack of sensation and the necessary general relaxation for all surgical procedures with the maintenance of special reflexes and without any peripheral paralysis.* Meltzer and Auer (1913-14), in collaboration with Peck and Elsberg, employed the combination clinically in seven cases. The patients were given from 0.4-0.6 gram magnesium sulphate per kilo intramuscularly, which was less than half of the effective dose. The ether was administered by endotracheal insufflation. In no case did the patients retain a spark of consciousness even when the concentration of ether in the insufflation was about one-sixth of the concentration usually employed. In all the cases there was complete relaxation of the muscles and in no case was there even the slightest indication of pain while operating in deep, sensitive places, as for example in radical breast amputations or radical removal of extensive, deeply located gland swellings in the neck or laparotomies with manipulation of the parietal peritoneum or gall bladder. With small doses of magnesium sulphate and very lightly maintained ether insufflation, there was often present a certain degree of lid reflex, even at times a spontaneous movement of the lids, without the slightest reaction to the operation on the part of the patient. In such cases the patient awoke shortly after the interruption of the administration of ether and the removal of the insufflation tube, but he could in no way remember the proceedings during the operation.

In order to determine in a quantitative way the extent of the synergism between magnesium sulphate and ether we have applied a technic described by Hooper and Becker, 1924, in which the drugs are injected intravenously into albino rats kept under uniform conditions. We have found that the intravenous administration of less than one-half the anesthetic dose of magnesium sulphate combined with approximately one-ninth the anesthetic dose of ether produces surgical anesthesia without increasing the toxicity of the combination.

EXPERIMENTAL PROCEDURE

Healthy, nonpregnant albino rats weighing approximately 150 grams were employed. The rats were obtained from one source and were kept on a constant well-balanced diet* for at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs when they were weighed and the doses administered per kilo body weight. Water was supplied during the fast period. The solutions of the drugs in distilled water were injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 c.c. The anesthetic dose was considered to have been reached when the application of a pencil point to the eyeball caused no reflex action of the eyelids. The injection was continued at the same rate until the animal stopped breathing when it was assumed the minimal lethal dose had been reached.

TABLE I

ANESTHETIC AND MINIMAL LETHAL DOSES OF ETHER AND MAGNESIUM SULPHATE ALONE OR IN COMBINATION WHEN INJECTED INTRAVENOUSLY INTO ALBINO RATS IN 6 PER CENT AQUEOUS SOLUTIONS

		ANESTHETIC DOSE		MINIMAL LETHAL DOSE	
			Average		Average
		mg. per kg.	mg. per kg.	mg. per kg.	mg. per kg.
Ether	6.0%	646		1985	
NaCl	0.6%	887		2112	
		627	744	1742	1892
		706		1853 (recovered)	
		693		1755	
		905		1905 (recovered)	
$MgSO_4$	6.0%	180-190 (10 rats)	185	190 (10 rats)	190
Ether	6.0%	180 (90 of each)		316 (158 of each)	
$MgSO_4$	6.0%	170 (85 of each)	170.8	392 (196 of each)	344.4
		192 (96 of each)		346 (173 of each)	
		140 (70 of each)	(85.4 of each)	344 (172 of each)	(172.2 of each)
		172 (86 of each)		324 (162 of each)	

It will be seen from the table that the anesthetic dose of 6.0 per cent ether is 744 milligrams per kilo body weight and the minimal lethal dose 1892 milligrams. The anesthetic dose is 39.3 per cent of the minimal lethal dose.

The anesthetic dose of 6.0 per cent magnesium sulphate is 185 milligrams per kilo body weight and the minimal lethal dose 190 milligrams. The anesthetic dose is 97.4 per cent of the minimal lethal dose.

The anesthetic dose of a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate is 170.8 milligrams and the minimal lethal dose is 344.4 milligrams. The anesthetic dose is 49.6 per cent of the minimal lethal dose.

Synergism may be defined as the reciprocal augmentation of the action of one drug by that of another. This augmentation is not due to a simple

*Hygienic Laboratory (U. S. P. H. S.) diet. A mixture composed of whole wheat flour, 53.5 per cent; corn meal, 10 per cent; skim milk powder, 33 per cent; calcium carbonate, 0.5 per cent; sodium chloride, 1 per cent; and cod liver oil, 2 per cent.

summation of similar pharmacologic actions because the effect produced is altogether too pronounced.

The table shows that the anesthetic action of ether and magnesium sulphate is definitely synergistic when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate. The anesthetic dose, 170.8 milligrams, contains 85.4 milligrams of ether and magnesium sulphate respectively in combination; 85.4 milligrams ether is 11.5 per cent of the anesthetic dose of straight 6.0 per cent ether (744 milligrams) and 85.4 milligrams magnesium sulphate is 46.2 per cent of the anesthetic dose of straight 6.0 per cent magnesium sulphate (185 milligrams). The total per cent is 57.7 per cent of the combined calculated anesthetic dose. The anesthetic dose of the combination, 170.8 milligrams, represents only 57.7 per cent of the calculated amount required for anesthesia when the drugs are administered singly. In other words there has been a reciprocal potentiation of the anesthetic action by combining magnesium sulphate and ether amounting in this case to approximately 73.5 per cent. We obtained anesthesia with 170.8 milligrams per kilo body weight of the combination. If magnesium sulphate and ether were not synergistic in producing anesthesia, a dose of 296 milligrams would have been required.

The toxicity of ether and magnesium sulphate is apparently additive when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate. The minimal lethal dose 344.4 milligrams contains 172.2 milligrams of ether and magnesium sulphate respectively in combination; 172.2 milligrams ether is 9.1 per cent of the minimal lethal dose of straight 6 per cent ether (1892 milligrams) and 172.2 milligrams magnesium sulphate is 90.6 per cent of the minimal lethal dose of straight 6 per cent magnesium sulphate (190 milligrams). The total per cent is 99.7 per cent of the combined calculated minimal lethal dose. The toxicity of ether and magnesium sulphate in combination is not potentiated. It is a simple summation of the toxicities of the two compounds. The conclusion may therefore be drawn that ether and magnesium sulphate act synergistically in producing anesthesia without producing any increase in toxicity.

II. LOCAL REACTIONS FOLLOWING THE HYPODERMIC INJECTION OF CONCENTRATED MAGNESIUM SULPHATE SOLUTIONS

Meltzer and Auer, 1905, and others state that the subcutaneous injection of concentrated magnesium sulphate solutions into animals may produce abscesses. They further state that in their experiments no aseptic precautions were taken. In our experiments it has been found that abscesses may be avoided by the employment of sterile solutions of chemically pure magnesium sulphate, with asepsis at the time of the injection. We have injected sterile 25 or 50 per cent water solutions subcutaneously or intramuscularly into rats approximately 350 times and have followed over 10,000 hypodermic

injections of the ampuled solution (2 c.c. 50 per cent solution) into human beings without the production of a single abscess or severe tissue reaction.

BACTERIOLOGIC TESTS.—The following bacteriologic tests show that 25 per cent or 50 per cent solutions of magnesium sulphate have little or no germicidal action and that contaminated solutions are not readily sterilized by boiling.

A heavy suspension of an old broth culture of spore-bearing organisms originally obtained from soil was made in ten cubic centimeters of distilled water.

1. Control. One cubic centimeter of the bacterial suspension was added to fifty cubic centimeters of distilled water and thoroughly mixed. The diluted bacterial suspension was placed in resistant glass ampules and sealed. The ampules were then submerged in boiling water 120 minutes (2 hours). Dextrose broth and dextrose agar cultures were then made from the ampuled solution and incubated at 37° C.

Result.—Heavy growth in all tubes within forty-eight hours.

2. One cubic centimeter of the bacterial suspension was added to fifty cubic centimeters of a 25 per cent magnesium sulphate solution in distilled water and thoroughly mixed. The mixture was placed in resistant glass ampules and sealed. The ampules were then submerged in boiling water, five ampules each, for fifteen, thirty and 120 minutes respectively. Dextrose broth and dextrose agar cultures were then made from the ampuled solution and incubated at 37° C.

Result.—Heavy growth in all tubes within forty-eight hours.

3. One cubic centimeter of the bacterial suspension was added to fifty cubic centimeters of a 50 per cent magnesium sulphate solution in distilled water and thoroughly mixed. The mixture was placed in resistant glass ampules and sealed. The ampules were then submerged in boiling water, five ampules each, for fifteen, thirty and 120 minutes respectively. Dextrose broth and dextrose agar cultures were then made from the ampuled solution and incubated at 37° C.

Result.—Heavy growth in all tubes within forty-eight hours.

As shown above, the spore-bearing organisms used in this test withstand boiling temperature (100° C.) under slight presure for at least two hours in the presence of either a 25 per cent or a 50 per cent water solution of magnesium sulphate.

III. THE SIMULTANEOUS ADMINISTRATION OF MAGNESIUM SULPHATE AND MORPHINE

The combination of magnesium sulphate with morphine, in anesthesia, was first suggested by Pellini, who in association with Gwathmey, 1921, determined the effect of this combination by carrying out a sufficient number of dog experiments to prove that one-eighth grain of morphine dissolved in 2 c.c. of a 25 per cent sterile solution of chemically pure magnesium sulphate, given by hypodermic injection and repeated twice at half-hour intervals, analgized the animals sufficiently for a full force of an artery clamp to be placed anywhere on the skin without producing any sign of pain. The control animals given the same amount of morphine in plain water were not analgized to anything like this degree.

After numerous clinical observations, Gwathmey established the fact that morphine may be given in a 50 per cent sterile solution of chemically pure magnesium sulphate instead of plain water and that in this combination the therapeutic value of morphine is increased from 50 to 100 per cent. To illustrate: one-eighth grain of morphine dissolved in 2 c.c. of sterile 50 per cent

magnesium sulphate solution, intramuscularly injected, will relieve pain for from ten to thirty hours, as compared with two to four hours with morphine alone. He states that magnesium sulphate when used as a synergist, exerts no deleterious effect on any of the tissues or organs of the body, including the respiratory center. It seems to act mechanically with morphine, holding it in contact with the tissues longer than the morphine alone is able to maintain such contact. With ether, and with nitrous oxide and oxygen it acts by deepening or increasing the effect, rather than by prolonging it. When magnesium sulphate is used with ether, the latter may be cut one-third to one-half in amount. When magnesium sulphate is used with nitrous oxide and oxygen, the oxygen may be considerably increased and the nitrous oxide decreased.

Gwathmey, 1924, holds that the difficulties experienced with the usual inhalation anesthesia are the lack of relaxation essential for many surgical procedures, and the after-effects of nausea, vomiting, and gas pains, induced by morphine when used alone. By the addition of magnesium sulphate to the preliminary morphine medication, a better relaxation is obtained and the nausea, vomiting, and gas pains are much reduced, if not entirely eliminated. If on the other hand, no allowance is made by the anesthetist, and if a third stage anesthesia is superimposed upon the analgesia induced by preliminary medication, the patient is immediately plunged into the danger zone and possibly into a condition of shock from the inhalation anesthetic. The same degree of relaxation can be obtained with perfect safety by maintaining the first stage of inhalation anesthesia, thus removing the patient from the danger zone by the second and third stages of anesthesia, accomplishing analgesia with unconsciousness. This condition is approximated by using morphine with magnesium sulphate as the analgesic, and inducing unconsciousness with nitrous oxide, ethylene and oxygen.

The hypodermic injection of morphine is occasionally followed by some nausea, which is much more frequently present during recovery from the drug. Evidently the effect of the magnesium sulphate abolishes this delayed untoward effect of morphine. It is well known that the preliminary administration of morphine abolishes the stage of excitement and is a potent factor of safety, since over 90 per cent of all deaths occur in the first five minutes of any inhalation anesthesia. The preliminary injection of morphine renders the anesthesia easier, smoother, and safer. The nausea and vomiting come on later as the effect is wearing off, and are sometimes most disastrous. By using magnesium sulphate with morphine, the morphine effect is retained during the time the patient is emerging from the inhalation anesthesia, and nausea and vomiting are eliminated, as a state of analgesia still exists. Wound and gas pains are likewise reduced to a minimum and quite often are entirely eliminated.

The following case from A. V. S. Lambert's service of the Presbyterian Hospital, New York, illustrates the difference between magnesium sulphate and plain water when used with morphine.

"No. 46241 F.—Badly lacerated wound of leg, gas infection, opened wide December first, gastrocnemius severed.

December		Duration, hours
2nd.	1:15 A.M. Morphine 1/5, $MgSO_4$ 2 c.c. 25%, quiet until 5	5
	6:15 A.M. Morphine 1/12, $MgSO_4$ 2 c.c. 25%, no pain all day	18
	The magnesium was now discontinued. Morphine alone used.	
3rd.	1:30 P.M. Morphine 1/6, pain in two hours	2
	3:25 P.M. Morphine 1/6, pain at 6:40 P.M.	3¼
	The magnesium was again added to the morphine.	
	6:55 P.M. Morphine 1/6, $MgSO_4$ 25%, 2 c.c. no pain till next day	23
4th.	5:00 P.M. Morphine 1/10, $MgSO_4$ 2 c.c. 25%, no pain till next A.M.	17
5th.	10:15 A.M. Morphine 1/10, $MgSO_4$ 3 c.c. 25%, quiet all day	10
	8:15 P.M. $MgSO_4$ 3 c.c., very good night	19
6th.	3:50 P.M. $MgSO_4$ 3 c.c. 25%, severe pain after 3 hours	3
	6:15 P.M. Morphine 1/8, $MgSO_4$ 2 c.c. 25%, jerking of leg in 2 hours	2
	9:15 P.M. Morphine 1/8, $MgSO_4$ 2 c.c. 25%, quiet night	19

The patient required one hypodermic of morphine 1/10 to 1/6 and $MgSO_4$ 2 c.c. 25 per cent each day after this until December 13. Codeine and morphine alone did not give relief."

From the above protocol it may be concluded that when the magnesium sulphate (2 to 4 c.c. of a 25 per cent chemically pure solution) is used with morphine, the value of the morphine is increased 50 to 100 per cent. By either increasing or repeating the dose of morphine with sterile water, its action is intensified but not prolonged, as when used with magnesium sulphate.

Smythe, 1923, employed three hypodermic injections of one-eighth grain of morphine dissolved in 2 c.c. of a 25 per cent solution of magnesium sulphate given at twenty minute intervals as a preliminary to novocaine and nitrous oxide-oxygen anesthesia, for general operations. He states that the patient is neither frightened nor apprehensive concerning the operation or its outcome. The stage of induction is greatly shortened and there is rarely a period of excitement. Forty per cent of the patients thus prepared did not require an analgesic after operation. The record of the nurse and bedside notes show that the patients go on an average four times as long after an operation before an analgesic is required as patients who are given morphine dissolved in sterile water alone. The appetite returned earlier in patients operated upon when magnesium sulphate had been employed, because of the absence of pain and restlessness incident to the trauma inflicted at operation.

King, 1923, recommends two or three hypodermic injections of one-eighth grain of morphine dissolved in 2 c.c. of the 25 per cent solution of magnesium sulphate repeated at one-half hour intervals as a preliminary medication for novocaine anesthesia in tonsillectomies. He states that the magnesium sulphate in combination with the morphine produces an analgesic action, so that a small amount of the local anesthetic is very effective.

Weston and Howard, 1923, have injected 2 c.c. or more of a 50 per cent solution of the salt without morphine subcutaneously and intramuscularly more than a thousand times in mental cases with no local pain or sloughing. They state the sedative action occurs in fifteen to thirty minutes and the patient sleeps five to seven hours. In a few instances the patient became quiet but did not sleep. It was found to be a very good substitute for mor-

phine and hyoscine. In 82.7 per cent of the cases it was effective. In 6 per cent the dose was repeated before sedation occurred. In 11 per cent no effect was noted even after three or more doses. They further state the salt is quite harmless in the dose necessary to produce sedative effect and can be given liberally when necessary.

Lederer, 1924, in an article entitled synergistic analgesia as employed in the first 1000 cases about the head and neck, advocates the use of three intramuscular injections of one-eighth grain of morphine dissolved in 2 c.c. of a 25 per cent solution of magnesium sulphate with a rectal instillation containing equal parts of olive oil and ether (each 3 ounces) and 2 drams of paraldehyde. The hypodermic injections were given at thirty minute intervals beginning three to four hours prior to operation. The rectal instillation was begun thirty minutes after the last hypodermic injection. Supplementary anesthesia, either by local infiltration or by drop ether was required in 12 per cent of the cases. There was no excitement stage present during the induction of the analgesia. Time in regard to the duration of the operation was a negligible factor, as the narcosis was smooth and of uniform depth for hours. At no time was there cyanosis or loss of body heat. Recovery from the analgesia was rapid; was not followed by lack of appetite; nor was there any tendency to mental depression. Postoperative medication was required in less than 0.5 per cent of the cases and then only in small amounts. Signs of irritation of the gastrointestinal tract or respiratory tract were never present. There were no kidney or bladder disturbances. One patient received the analgesic fourteen times in the course of three years without noticing any untoward symptoms or drug tolerance.

Gwathmey and his collaborators, 1923 and 1924, are perfecting a synergistic method for inducing obstetric analgesia. The procedure is based in great part upon the synergistic action of magnesium sulphate and ether. The technic, developed after experience with over 1000 cases, calls for three hypodermic injections and one rectal instillation. The first hypodermic injection is given when the pains are four or five minutes apart and lasting thirty or more seconds. It consists of one-sixth grain of morphine, dissolved in 2 c.c. of a 50 per cent solution of magnesium sulphate. The morphine is not repeated. The rectal instillation which follows within twenty minutes, if no relief is afforded by the first hypodermic injection, contains quinine hydrobromide, 20 grains; alcohol, 3 drams; ether, 2½ ounces, and olive oil, q. s. ad 4 ounces. If the first hypodermic injection produces a marked sedative effect, the instillation is delayed from one to two hours. The effect of the hypodermic and instillation continues for about four hours, but if insufficient, one or two additional hypodermic injections of 2 c.c. 50 per cent magnesium sulphate solution are given to deepen the effect. The instillation may also be repeated after four hours. Pain is eliminated in over 90 per cent of the cases, labor is not delayed, and memory of events is either clouded or completely obliterated. The condition of the baby is not affected by the medication. It is the exception to have delayed respiration.

Adams, 1924, employed one or two intramuscular injections of one-fourth

grain morphine dissolved in 2 c.c. of a 25 per cent solution of magnesium sulphate as an obstetric analgesia in sixty cases. The initial injections were made into the deltoid muscle when the cervix was on the average two and one-half centimeters dilated. In thirteen cases where the effect of the medication seemed favorable but not lasting, the injections were repeated. The average dilatation at the time of the second injection was four and one-half centimeters. There was a definite sedative effect on the labor pains in the large majority of cases (93 per cent) without increasing the fetal or maternal morbidity or mortality. The total length of labor was not prolonged. Postpartum bleeding was not excessive. The uterus at all times retained a good tone and no abnormal tendency to relaxation was noted. The author concludes that the treatment possesses possibilities as a simple, inexpensive and apparently harmless method of obstetric analgesia.

IV. ANALGESIC, ANESTHETIC AND TOXICITY EXPERIMENTS WITH MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND MORPHINE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, MORPHINE AND NOVOCAINE

In order to make our experiments of practical value, and for comparative purposes, the following sterile solutions were employed in the subcutaneous experiments:

1. Magnesium sulphate 50 per cent.
2. Magnesium sulphate 50 per cent with morphine sulphate 0.4 per cent (2 c.c. contains one-eighth grain morphine).
3. Magnesium sulphate 50 per cent with novocaine 2.5 per cent.*
4. Magnesium sulphate 50 per cent with morphine 0.4 per cent and novocaine 2.5 per cent.

In the intravenous experiments the above solutions were diluted with a sufficient quantity of distilled water to make a 6 per cent magnesium sulphate content.

In the computation of the amount of solution to be injected at a given dose the magnesium sulphate content of the solution alone was considered. The amount of novocaine and morphine contained in the solutions was disregarded.

SUBCUTANEOUS EXPERIMENTS WITH ALBINO RATS

Healthy, nonpregnant albino rats weighing between 100 and 150 grams were employed. The rats were obtained from one source and were kept on the Hygienic Laboratory (U.S.P.H.S.) diet at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs when they were weighed and the doses were administered per kilo body weight. Water was supplied during the fast period. The hair over the back above the tail was clipped and the injections were

*In the preliminary subcutaneous experiments in which a 50 per cent water solution of magnesium sulphate was used alone and in combination with 1.25 per cent, 2.5 per cent, 5 per cent, and 10 per cent novocaine, it was found that the most suitable combination for the production of analgesia and anesthesia was a 50 per cent magnesium sulphate solution with 2.5 novocaine. The latter combination was therefore adopted for the more detailed experiments.

made subcutaneously in the left lumbar region. The animals were kept under observation until they completely recovered. The analgesic dose was considered to have been reached when the corneal and tail reflexes were absent or markedly diminished with the animal otherwise normal or somewhat de-

TABLE II

COMPARISON OF THE ANALGESIC AND ANESTHETIC DOSES OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND MORPHINE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, MORPHINE AND NOVOCAINE. ALBINO RATS INJECTED SUBCUTANEOUSLY

	DOSE	RESULTS OBTAINED
	mg. per kg.	
$MgSO_4$ 50.0%	400	Active and normal. Corneal and tail reflexes maintained
	800	Active and normal. Corneal and tail reflexes maintained
	1000	Depression within 20 minutes lasting approximately 25 minutes. Corneal and tail reflexes maintained. Does not assume dorsal position
	1000	Some depression within 27 minutes lasting approximately 19 minutes. Corneal reflex somewhat diminished. Tail reflex maintained. Does not assume dorsal position
	1200	*Complete analgesia within 20 minutes. Corneal and tail reflexes absent 12 minutes. Does not assume dorsal position*
	1400	*Complete anesthesia with dorsal position, relaxation and absence of corneal and tail reflexes within 14 minutes lasting approximately 28 minutes*
$MgSO_4$ 50.0% Morphine Sulphate 0.4%	400	Active and normal. Corneal and tail reflexes maintained
	600	Active and normal. Corneal and tail reflexes maintained
	800	*Definite analgesia within 23 minutes. Corneal and tail reflexes absent approximately 15 minutes. Does not maintain dorsal position*
	1000	*Complete analgesia within 15 minutes and complete anesthesia within 21 minutes. Corneal and tail reflexes absent approximately 70 minutes. Maintained dorsal position approximately 30 minutes*
$MgSO_4$ 50.0% Novocaine 2.5%	600	Active and normal. Corneal and tail reflexes maintained
	800	*Definite analgesia within 19 minutes. Corneal and tail reflexes absent or markedly diminished for approximately 30 minutes. Does not maintain dorsal position*
	1000	*Complete anesthesia, with dorsal position, relaxation, automatic breathing and absence of corneal and tail reflexes within 20 minutes lasting approximately 30 minutes. Complete recovery within 1 hour*
$MgSO_4$ 50.0% Novocaine 2.5% Morphine Sulphate 0.4% (⅛ grain of morphine in 2 c.c. solution)	200	Active and normal. Corneal and tail reflexes maintained
	400	Definite analgesia within 32 minutes. Corneal and tail reflexes markedly diminished approximately 30 minutes
	600	*Complete analgesia within 25 minutes. Corneal and tail reflexes absent approximately 40 minutes*
	800	*Complete analgesia within 24 minutes and complete anesthesia within 30 minutes. Corneal and tail reflexes absent approximately 72 minutes. Remained in dorsal position 25 minutes*
	1000	Complete analgesia within 20 minutes and complete anesthesia within 32 minutes. Corneal and tail reflexes absent approximately 130 minutes. Maintained dorsal position 40 minutes

pressed. The anesthetic dose was considered to have been reached when the animal maintained a dorsal position with relaxation, automatic respiration and absence of corneal and tail reflexes. The corneal reflex was considered absent when the application of a pencil point to the cornea caused no reflex action of the eyelids. The tail reflex was obtained by applying a mouse

tooth forceps to the base of the tail with sufficient force to prick the skin. A normal animal will invariably swing around and bite the forceps.

Animal experiments dealing with drug combinations are difficult, usually unsatisfactory, and in order to draw accurate conclusions a large number of well-controlled experiments are required. In Table II, eighteen representative experiments are tabulated. Approximately two hundred and seventy-five experiments of this type have been carried out.

In the first column are given the solutions employed; in the second column the dose in milligrams per kilo body weight; in the third column a summary of the effects produced.

Table II shows the analgesic dose of magnesium sulphate for the albino rat, when injected in 50 per cent water solution, is approximately 1200 milligrams per kilo and the anesthetic dose 1400 milligrams.

The analgesic dose of 50 per cent magnesium sulphate with 0.4 per cent morphine sulphate is 800 milligrams and the anesthetic dose 1000 milligrams per kilo.

The analgesic dose of 50 per cent magnesium sulphate with 2.5 per cent novocaine is also 800 milligrams and the anesthetic dose 1000 milligrams.

The analgesic dose of 50 per cent magnesium sulphate solution with 0.4 per cent morphine and 2.5 per cent novocaine is between 400 and 600 milligrams and the anesthetic dose 800 milligrams. The addition of 2.5 per cent novocaine to the magnesium sulphate solution with morphine (one-eighth grain morphine in 2 c.c. solution) increases the analgesic and anesthetic actions of the combination at least 25 per cent and also definitely prolongs the effect.

TABLE III

COMPARISON OF THE TOXICITY OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, MORPHINE AND NOVOCAINE. RATS INJECTED SUBCUTANEOUSLY. PERCENTAGE OF RATS THAT DIED

	DOSE EXPRESSED IN MILLIGRAMS PER KILO BODY WEIGHT							
	1100	1200	1300	1400	1500	1600	1800	2000
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
$MgSO_4$ 50.0%					0(5)	20(5)	20(5)	20(5)
$MgSO_4$ 50.0% Novocaine 2.5%		0(5)	0(5)	20(5)	60(5)			
$MgSO_4$ 50.0% Novocaine 2.5% Morphine 0.4%	0(5)	80(5)	100(5)	100(5)	100(5)			

The numbers within the parentheses represent the number of rats used in the series.

The technic of the experiments presented in Table III was the same as that used for the experiments tabulated in Table II except that a series of rats was run at each dose, regardless of analgesia or anesthesia. The animals were kept under observation for twenty-four hours, during which time they were fed the routine diet.

It will be seen from Table III that the maximal tolerated dose (minimal dose at which any of a series of animals dies) is 1600 milligrams per kilo for the 50 per cent magnesium sulphate solution; 1400 milligrams for the 50

per cent magnesium sulphate solution with 2.5 per cent novocaine; and 1200 milligrams for the 50 per cent magnesium sulphate solution containing 2.5 per cent novocaine and 0.4 per cent morphine. These results are based upon subcutaneous injections. They are in marked contrast with the results later presented in this article, where the solutions were injected intravenously and the toxicity found to be approximately the same. The discrepancy between

TABLE IV

COMPARISON OF THE ANALGESIC DOSE OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND MORPHINE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, NOVOCAINE AND MORPHINE. RATS INJECTED INTRAVENOUSLY

		ANALGESIC DOSE		DURATION OF ANALGESIA	REMARKS
			AVERAGE		
		mg. per kg.	mg. per kg.	minutes	
$MgSO_4$	6.0%	180-190	185 (15 rats)	1-2	
$MgSO_4$	6.0%	43			Solution in proportion to that used in the subcutaneous experiments
Morphine Sulphate	0.048%	64 40 39 34 32 56 33 44 44	42.9 (10 rats)	10-45	
$MgSO_4$	6.0%	65			Solution in proportion to that used in the subcutaneous experiments
Novocaine	0.3%	57 67 70 44 43 32 57 53 77 43 59 65 43 43	54.5 (15 rats)	2-5	
$MgSO_4$	6.0%	40			
Novocaine	0.3%	36			Solution in proportion to that used in the subcutaneous experiments
Morphine Sulphate	0.048%	24 30 40 33 27 40 30 34 34 29 31 32 22 26 42 36 30 33	32.4 (20 rats)	15-60	

the subcutaneous and intravenous experiments may possibly be due to difference in the rate of absorption of the solutions from the subcutaneous tissues. In our opinion the intravenous experiments are much more reliable.

INTRAVENOUS EXPERIMENTS WITH ALBINO RATS

In the following experiments the technic was the same as in the previous experiments except that the solutions of the drugs in distilled water were injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 c.c. The analgesic dose was considered to have been reached when the application of a pencil point to the cornea caused no reflex action of the eyelid.

In carrying out the toxicity experiments, five rats were injected at each dose regardless of analgesia or anesthesia. The animals were kept under observation for twenty-four hours after injecting the solutions and were fed the routine diet.

Table IV shows that the analgesic dose of 6 per cent magnesium sulphate solution injected into the saphenous vein of the albino rat is approximately 185 milligrams per kilo. The analgesic dose of 6 per cent magnesium sulphate solution with 0.048 per cent morphine is 42.9 milligrams. The analgesic dose of 6 per cent magnesium sulphate solution with 0.3 per cent novocaine is 54.5 milligrams. The analgesic dose of 6 per cent magnesium sulphate solution with 0.3 per cent novocaine and 0.048 per cent morphine is 32.4 per cent. Further, the addition of the morphine prolongs the effect.

TABLE V

A QUANTITATIVE COMPARISON OF THE TOXICITY OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, NOVOCAINE AND MORPHINE. RATS INJECTED INTRAVENOUSLY. PERCENTAGE OF RATS THAT DIED

		DOSE EXPRESSED IN MILLIGRAMS PER KILO BODY WEIGHT		
		180	190	200
		per cent	per cent	per cent
$MgSO_4$	6.0%	0(5)	100(5)	80(5)
$MgSO_4$	6.0%			
Novocaine	0.3%	0(5)	20(5)	20(5)
$MgSO_4$	6.0%			
Novocaine	0.3%			
Morphine sulphate	0.048%	0(5)	60(5)	80(5)

The numbers within the parentheses represent the number of rats used in the series.

It will be seen from Table V that the addition of 0.3 per cent novocaine to a 6 per cent water solution of magnesium sulphate or the addition of 0.3 per cent novocaine and 0.048 per cent morphine sulphate, does not increase the toxicity of a 6 per cent magnesium sulphate solution when injected into the saphenous vein of the albino rat at the rate of twelve to fifteen seconds for each 0.1 c.c.

EXPERIMENTS WITH DOGS AND CATS

Beckman of Marquette University School of Medicine (personal communication)* has found that the subcutaneous injection of 2 c.c. of 50 per

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cent magnesium sulphate solution simultaneously with 0.25 c.c. of a 4 per cent solution of morphine per kilo into dogs is followed by certain undesirable symptoms which he attributes to the magnesium sulphate. He states: "These dogs have been much more depressed when brought into the anesthetizing room than those given morphine alone; they are whining as though in distress; the rate of respirations is not changed but the expirations are jerky (there is almost an expiratory grunt); and in most cases there has been a quite profuse salivation." The dose of morphine employed, 0.25 c.c. of a 4 per cent solution per kilo, represents a dose of 10 milligrams (one-sixth grain) per kilo or 110 milligrams (one and three-fourth grains) for a dog weighing 11 kilos (24.2 lbs.). The dose of morphine is very large and out of all proportion to the amount of magnesium sulphate used. Beckman's experiments show definitely that the combined administration of magnesium sulphate and morphine in disproportionate amounts is not practicable and that it would no doubt prove decidedly dangerous in clinical application. The combination of magnesium sulphate and morphine as employed in our experiments (2 c.c. 50 per cent magnesium sulphate solution with one-eighth grain of morphine) is administered clinically almost constantly with no untoward results. Several thousand of these injections are now being given every month. Based on animal experiments a clinical dose of 2 c.c. 50 per cent magnesium sulphate solution administered subcutaneously or intramuscularly is at least 100 times removed from the fatal dose. Meltzer and Auer, 1905, demonstrated conclusively that dogs withstand by subcutaneous injection 1.5 grams magnesium sulphate per kilo without showing any distress or respiratory symptoms. The dose of 1.5 grams magnesium sulphate per kilo represents a dose of 33 c.c. of a 50 per cent magnesium sulphate solution for a dog weighing 11 kilos (24.2 lbs.) or 225 c.c. for a man weighing 75 kilos (165 lbs.). They further found that the subcutaneous injection of 2 grams magnesium sulphate per kilo produced a profound anesthesia and general paralysis which led sooner or later to a calm death without being preceded or accompanied by any symptoms of excitation.

We have administered eight intramuscular injections at twenty minute intervals of either 2 c.c. 50 per cent magnesium sulphate solution with 0.4 per cent morphine (one-eighth grain in 2 c.c.), or 2 c.c. 50 per cent magnesium sulphate solution with 0.4 per cent morphine and 2.5 per cent novocaine into dogs weighing between eleven and twelve kilos. Each animal received 8 grams magnesium sulphate (661 milligrams per kilo, average) with 1 grain of morphine. They showed no untoward symptoms and the respirations remained normal throughout the experiments. There was some salivation but no vomiting or defecation following the injections of magnesium sulphate with morphine alone. There was no salivation, vomiting or defecation following the injections of magnesium sulphate, morphine and novocaine. The animals became definitely analgized, remained calm and quiet for several hours and were completely recovered by the following morning. In other experiments we administered single intramuscular injections of 8 c.c. 50 per cent magnesium sulphate solution containing one-half grain of morphine

both with and without the novocaine into dogs weighing seven to twelve kilos. None of the animals showed any untoward symptoms. A striking feature has been the absence of the defecation and vomiting which usually follows the injection of simple morphine solution.

The subcutaneous or intramuscular injections of a fatal or nearly fatal dose of morphine in the cat (20 milligrams per kilo) has an exciting effect. The animal races about as if in extreme terror, with prominent eyes and widely dilated pupils scratching and clawing wildly. Sometimes there are even convulsions. In all of the experiments that Meltzer and his collaborators carried out with the injection of magnesium sulphate, not a single instance was observed in which this salt produced an increase of excitation; on the contrary any effect which it produced was invariably in the direction of a reduction of excitation or of its complete temporary or permanent abolition. With these facts in mind eight cats were injected intramuscularly, three with morphine alone (20 milligrams per kilo) and five with the morphine dissolved in 50 per cent magnesium sulphate solution with 2.5 per cent novocaine (800-1200 milligrams $MgSO_4$ per kilo). The three control cats developed a violent mania. The animals that received the morphine dissolved in magnesium sulphate and novocaine did not become violent and four of the animals soon became deeply anesthetized. The experiments show that magnesium sulphate is capable of reducing or completely inhibiting the symptoms of excitation which follow the injection of morphine alone in the cat.

SUMMARY

The synergistic action of magnesium sulphate and ether is described on a quantitative basis.

Magnesium sulphate and ether act synergistically in producing anesthesia when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate. The synergism is of the potentiated type.

The toxicity of magnesium sulphate and ether is additive when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate. The toxicity is a simple summation of the toxicities of the two compounds.

The anesthetic dose of magnesium sulphate approaches the fatal dose very closely when injected into the saphenous vein of the albino rat as a 6.0 per cent water solution or when injected subcutaneously as a 50 per cent water solution. The margin of safety is slight.

The anesthetic dose of ether represents approximately 40 per cent of the minimal lethal dose when injected into the saphenous vein of the albino rat as a 6.0 per cent water solution.

The anesthetic dose of magnesium sulphate and ether represents approximately 50 per cent of the minimal lethal dose when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent magnesium sulphate and 6.0 per cent ether.

Concentrated magnesium sulphate solutions have little or no germicidal action. Contaminated solutions are not readily sterilized by boiling.

Sterile 25 or 50 per cent water solutions of specially tested, chemically pure magnesium sulphate do not produce abscesses or severe tissue reactions when injected with asepsis subcutaneously or intramuscularly into the albino rat or the human being (2 c.c. injected at one site).

A review of the literature dealing with the simultaneous administration of magnesium sulphate and morphine is given.

Comparative analgesic, anesthetic, and toxicity experiments with magnesium sulphate alone; magnesium sulphate and morphine; magnesium sulphate and novocaine; and magnesium sulphate, morphine and novocaine are given in which the drugs were injected subcutaneously and intravenously into the albino rat.

The addition of 2.5 per cent novocaine to a 50 per cent magnesium sulphate solution with morphine sulphate 0.4 per cent (2 c.c. contains one-eighth grain morphine) increases the analgesic and anesthetic actions of the combination at least 25 per cent and also definitely prolongs the effect when injected subcutaneously or intravenously into the albino rat.

In a limited number of experiments with dogs the subcutaneous or intramuscular administration of morphine dissolved in 50 per cent magnesium sulphate solution with 2.5 per cent novocaine (one-eighth grain morphine in 2 c.c.) has not been followed by salivation, vomiting or defecation.

The simultaneous administration of magnesium sulphate 50 per cent solution with 2.5 per cent novocaine (800-1200 milligrams magnesium sulphate per kilo) reduces or completely inhibits the symptoms of excitation which follow the subcutaneous injection of a fatal or nearly fatal dose of morphine in the cat.

Based on animal experiments the clinical dose of 2 c.c. 50 per cent magnesium sulphate solution administered subcutaneously or intramuscularly is at least 100 times removed from the fatal dose.

Magnesium sulphate used alone in sufficient amount to produce anesthesia is the most dangerous anesthetic known. When used as a synergist with other drugs it may become one of the most valuable drugs in the pharmacopeia.

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ACUTE MYELOID LEUCEMIA*

(Report of a case)

By A. A. Blair, M.D., Fort Smith, Arkansas

THE incidence of acute myeloid leucemia is exceedingly rare. In fact we are skeptical in accepting our findings, often in making a diagnosis, owing to the few cases reported and variability of blood findings. However, during the past few years sufficient clinical and microscopic data has been obtained to clear up this doubt. Clinically it is characterized by sudden onset, short and stormy course, hyperpyrexia, rapid and progressive anemia, usually infective and ulcerative changes of the oral cavity with frequent hemorrhages from the mucous membranes, terminating in death in from four weeks to four months.

The suggestion that it is of infectious origin is well understood, being frequently associated with dental infection, ulcerative tonsillitis, etc. According to Gorham† to make a diagnosis of the acute form of this disease, the patient should present specific findings, beginning with a subleucemic stage, later development of a characteristic blood picture of myeloblasts and myelocytes and certain transition forms (proving the myeloid elements by the oxidase reaction), with typical gross and histologic findings and early death. Without going into discussion of all the phases of the disease, I wish to present a case, certainly one of the above type.

Patient, M. A., consulted us first July 14, 1924. White female, age fifty-three (married thirty-four years), three children living, one miscarriage. Previous history of no importance. Clinical history shows that she had influenza in February this year, but has been in fair health until present attack. Three weeks prior to July 14, she had six teeth extracted by her dentist. Since this time her gums have been badly infected and she has had pain in her face and neck, with elevation of temperature, pain in her arms and legs and progressive weakness, menorrhagia and metrorrhagia. Her first examination of July 14, 1924, revealed the following briefly:

A small sallow individual 51 inches in height, 91 pounds. Blood pressure 120/64; temperature 100° F.; cheeks pale; hair on head and pubis scanty; mouth showed marked bad dental sepsis, gums swollen and bleeding occasionally, enlargement of cervical glands; skin over chest and part of abdomen shows numerous small intracutaneous nodules about the size of a pea. Heart examination negative. Chest showed a few moist râles in left apex, not persistent; expansion good. Abdomen: Spleen palpable. Vaginal examination: Uterus slightly

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†Gorham, L. W.: Acute Myelogenous Leucemia, Albany Med. Ann., 1917, xxxviii, 201

enlarged, and soft, old cervical laceration present. Reflexes were normal. Laboratory examination: Urine showed a heavy cloud of albumin; many granular casts, red and pus cells; sputum negative; stool negative; blood Wassermann negative; blood culture negative; malaria negative; hemoglobin 39 per cent (Sahli). R. B. C. 2,000,000; total white count 24,400; differential count, polymorphonuclear neutrophile 23 per cent; small lymphocytes 10 per cent; large lymphocytes 2 per cent; eosinophiles 2 per cent; neutrophilic myelocytes 35 per cent; myeloblasts 25 per cent; smears obtained from ulcerations of gums showed numerous fusiform bacilli and spirilla. Patient was ordered to Sparks Memorial Hospital for observation and treatment. Arsenic in the form of sodium cacodylate and Fowler's solution was pushed. Cleansing solution for mouth was given.

Progress, July 16, 1924, patient appeared some better. Temperature 100° F. axillary; not so much pain in face; blood culture negative.

July 17. Temperature 100.2° F. Condition about the same. Spleen on level of umbilicus.

July 18 to 24. No noticeable improvement during this time. X-ray treatment over spleen and long bones given every third day.

July 25. Temperature rose to 103° F.; pulse 120. Patient felt very uncomfortable. Sense of choking in throat, bleeding from the gums, pain in face; ¼ gr. morphine given. Blood culture negative; stool examination positive for blood.

July 26. Temperature 104° F. Spleen could be easily felt by dipping fingers over crest of ileum, lower border three finger width below umbilicus in median line, rapidly filling left side of abdomen. Some apparent enlargement of the liver; tender over shafts of long bones; white blood count 35,000; red blood count 1,800,000; hemoglobin 33 per cent; polymorphonuclear neutrophile 22 per cent; small lymphocytes 11 per cent; large lymphocytes 1 per cent; myelocytes, neutrophilic 36 per cent; myeloblasts 31 per cent. Predominating myelocytes and myeloblasts being from 20 to 23 microns, the oxidase reaction not performed.

The temperature between July 26 and Aug. 3 ranged from 102° F. to 104° F.; pulse 120 to 150. Patient nauseated and vomiting. Pain in face and limbs only relieved by morphine; very nervous and tossing about in bed; rational only part of time.

Died at 5 P.M. Aug. 4, 1924. Permission for autopsy on this patient was not given.

LABORATORY METHODS

A SIMPLE THERAPEUTIC METHOD FOR THE STANDARDIZATION OF DIGITALIS*

By J. B. Berardi, Ph.G., B.S., M. C. Canan, B.S., and H. McGuigan, M.D.

THE available methods for the standardization of digitalis are far from satisfactory. The frog method which is most used requires several animals and a considerable amount of time to carry out the standardization. The results are frequently inconsistent and unsatisfactory. The cat method also often gives variable results, and as the standardization is done under an anesthetic, it is far removed from the condition in which digitalis is employed in the practice of medicine. The present investigation is an attempt to approximate therapeutic usage and at the same time devise a simple and available method. This procedure consists briefly in the intravenous injection of the drug into a normal dog without anesthesia. The heart rate is counted before and after the drug and the slowing of the rate is taken as a measure of the activity of the drug. If the preparation be of standard strength, 0.02 c.c. of the tincture per kilo weight of dog, will reduce his heart rate 20 per cent in from thirty to sixty minutes. The animal is not injured and may be used again for the same purpose in the course of a few days. The details of the method are as follows:

A dog is weighed. The normal heart rate is then determined by taking an average of four or five results at ten minute intervals. It is important to get an accurate count without excitement. A definite amount of tincture of digitalis diluted with physiologic salt solution is then given intravenously. After the injection the animal is carefully watched, the heart rate being taken every ten minutes until the greatest drop has been recorded and the heart shows signs of returning to normal. From this data the percentage drop is calculated. Observations upon respiration showed no marked variations, hence respiration is not considered in the standardization.

The first series of dogs were injected with ¼ c.c. of tincture diluted in salt solution. This was a dosage of from 0.017 to 0.055 c.c. per kilogram. We found, in seven dogs examined, that there was no uniform time before a change in the heart rate was observed, but that the percentage slowing showed a definite relation to the dosage per kilogram body weight and that the greatest slowing occurred between thirty and sixty minutes after injection. Some of these animals were used several times in order to determine the accumulative effect of the drug.

*From the Laboratory of Pharmacology, University of Illinois College of Medicine, Chicago, Illinois.
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DIGITALIS

	WEIGHT IN KILOS	DOSE IN C.C.	DOSE PER KILO	NORMAL HEART RATE	DIGITALIS HEART RATE	TIME FOR DROP	AMOUNT OF DROP IN BEATS	PER CENT SLOWING OF HEART	
TABLE I	14.09	1/4	0.017	107.5	68	30′	39.5	36	Male
	13.3	1/4	0.018	98.5	68	30′	30.5	30	Female
	6.8	1/4	0.036	124.5	76	19′	48.5	39	Male
	8.7	1/4	0.028	108.5	88	5′	20.5	19	Female
	4.5	1/4	0.055	178	110	10′	68	38	Female
	12.5	1/4	0.020	117.5	80	17′	37.5	31	Male
	11	1/4	0.022	104.7	88	2′	14.7	14	Male
TABLE II	12	1/2	0.041	137.5	120	5′	17.5	12.5	Female
	5.6	1/2	0.088	142	120	5′	22.1	15	Male
	5	1/2	0.10	130	72	30′	58	44	Female
TABLE III	20	1	0.05	82	59	1 hr.	23	27	Male
	20	1	0.05	104	80	1 " 44′	34	32	Male
	18.1	1	0.055	110	90	1 " 21′	30	27	Male
	11.5	1	0.086	109	76	33′	33	30.2	Male
TABLE IV	59	2	0.35	114	90	33′	24	22	Female
	11.5	2	0.18	109	80	50′	29	26	Male
TABLE V	15.4	3	0.19	105	72	1 hr. 35′	33	31	Female
	8.3	3	0.16	101	56	2 "	45	44	Male

DIGITALIS CONT'D

	WEIGHT IN KILOS	DOSE IN C.C.	DOSE PER KILO	NORMAL HEART RATE	DIGITALIS HEART RATE	TIME FOR DROP	AMOUNT OF DROP IN BEATS	PER CENT SLOWING OF HEART	
	7.1	.071	0.01	156	116	1 hr. 10′	39	24	Female
TABLE VI	17.2	.172	0.01	98	84	1 " 10′	14	14	Female
	17.4	.174	0.01	110	92	42′	18	18	Male
	16.7	.167	0.01	97	85	1 " 07′	12	12	Male
	10.94	.208	0.02	137	114	1 " 10′	33	24	Male
	15.9	.318	0.02	121	100	1 " 05′	21	21	Male
	21.8	.438	0.02	124	100	27′	24	19	Male
	26.8	.536	0.02	96	76	1 " 30′	20	20	Male
	10.7	.214	0.02	120	88	1 " 20′	32	23	Male
	9.3	.186	0.02	108	84	56′	24	22	Female
	9.8	.196	0.02	115	93	56′	22	20	Male
TABLE VII	14.5	.29	0.02	130	98	45′	32	17	Female
	17.5	.35	0.02	122	96	1 " 05′	26	22	Female
	10	.2	0.02	98	82	59′	16	26	Female
	17.3	.356	0.02	112	90	1 " 13′	22	19.6	Female
	23.6	.46	0.02	102.5	87	15′	15	15.2	Male
	5.8	.116	0.02	118	90	45′	28	23.6	Male
	14	.280	0.02	104	84	1 " 10′	20	19.2	Male
	18 8	.376	0.02	118	90	1 " 05′	28	23.8	Female
TABLE VIII	20.8	.624	0.03	128	88	55′	40	31.2	Female
	10.36	.310	0.03	118	92	1 " 35′	26	22	Female

Another series of animals were injected with increasing doses until toxic symptoms appeared. Doses which were used were as follows: from ½, 1, 2, and 3 c.c.

Doses of 0.03 c.c. or more per kilo body weight usually elicited toxic symptoms such as nausea and vomiting. This led us to think that the therapeutic dose, as far as dogs are concerned, must lie somewhere between 0.01 c.c. and 0.03 c.c. per kilo, which we found to be approximately 0.02 c.c. per kilogram body weight of dog. When this dose was used the percentage drop was rather constant and no toxic symptoms appeared. The 0.01 dose per kilo body weight did not produce the maximum effect. In seven dogs injected with the 0.02 c.c. of the tincture per kilogram body weight the percentage drop varied only 5 per cent. In eleven other dogs used, the dose was varied from 0.01 to 0.03 c.c. of tincture per kilo. In these experiments the percentage drop showed greater variations.

DISCUSSION

The method presented is based upon a therapeutic standard and requires very little time and expense.

From the results one is justified in saying that standardization may be secured from the use of a few dogs. After about one week these dogs can be used again for standardization, thus eliminating a great expense for new animals.

The most constant percentage drop, produced by the 0.02 c.c. per kilo body weight of dog, does not produce toxic effects and is well within the therapeutic limits. Too small doses do not give so constant a result, too large doses, because of the nausea, do not give reliable results. A medium sized dose (0.02 c.c. per kilo) presents a variation of about 5 per cent on either side of a 20 per cent reduction of heart rate. The method is presented tentatively with the belief that it is reliable within reasonable limits, and it can be used in many instances where more laborious methods are unavailable. With a single dog, without injuring him, one may closely approximate the strength of a given preparation since 0.03 c.c. per kilo of a standard tincture will usually cause nausea and vomiting and 0.01 c.c. will cause considerable slowing of the heart rate. If a group of dogs be kept for the standardization, very dependable results can be obtained. This method of administering digitalis is very impressive as a class exercise.

PROPOSED MODIFICATION OF THE KAISERLING METHOD FOR PRESERVATION OF SPECIMENS FOR DISPLAY PURPOSES*

By Richard Lundquist, and H. E. Robertson, M.D., Rochester, Minnesota

In April, 1924, this proposed method for preserving gross specimens was presented before the International Association of Medical Museums. Further experience with the technic, as given, has only strengthened our confidence in its efficiency, and fully justifies this second report.

Like most museum workers, we had formerly considered that the Kaiserling method, as published in 1899, was by far the most reliable method available for use. However, the Kaiserling method was rarely entirely satisfactory, having certain definite objectionable features, as follows:

1. A lack of penetrative power, which has often resulted in too long an exposure to the fixing solution in an attempt to obtain proper fixation. The color return in these specimens following the use of alcohol would not be as good as in those which had been exposed to the fixing solution for a shorter period.

2. The specimen showed an undue amount of distortion, even after being carefully arranged in the fixing solution. When large numbers of specimens are handled, it is almost inevitable that a certain number will be improperly placed in the fixing solution. It was found practically impossible to correct these distortions after the specimens had once become hardened.

3. Further hardening and stiffening of the tissues in the alcohol used for the restoration of color.

4. The extremely uncertain permanency of color preservation.

The first two objections were considered to result from too strong a percentage of formaldehyde in the fixing solution. This hardened the outer surface of the tissue exposed to the fluid to such an extent as to prevent proper penetration of fluid. In an attempt to overcome these difficulties the following formula was devised, and the use of alcohol thereby eliminated.

Solution 1, Fixing Solution

Potassium acetate	850 gm.
Potassium nitrate	450 gm.
Chloral hydrate	800 gm.
Formaldehyde (40 per cent gas)	4,445 c.c.
Water	40,000 c.c.

It is important that the specimens be placed in the fixing fluid as soon as possible, and that there be a comparatively large volume of this fluid. The

*From Section on Pathologic Anatomy, Mayo Clinic, Rochester, Minn.
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volume should be at least from ten to twelve times greater than that of the specimens. To make this possible, we use a stoneware crock with an inside diameter of 21 inches, an inside depth of 12 inches and a capacity of 66 liters (16.5 gallons). This crock gives the volume desired, and also furnishes the wide diameter which is very convenient when a number of specimens are prepared simultaneously. Formulas as given are the correct amounts for these crocks.* Specimens should not be allowed to lie against each other, or against the bottom of the container. To avoid this, we suspend the specimens by a string attached to a cork which has been previously boiled in paraffin. Care must be exercised to attach the string in such a manner as to allow the specimen to assume its natural shape. The use of the cork prevents the specimens from lying on the bottom of the jar, and the suspended specimens do not crowd each other. This procedure further facilitates the finding of any individual specimen when desired. The specimens should be thoroughly fixed in the first solution. The time required for this will vary with different specimens, and the worker will have to use his personal judgment. A good plan is to allow at least from twelve to twenty-four hours for thin membranes, such as the intestines, and forty-eight hours or more for slices of liver, spleen, kidney, and so forth. Specimens may be tested by using gentle pressure to see if any unfixed blood will exude from cut surfaces. However, the danger of loss of color, owing to too long exposure to fixing solution, is not nearly as great as with the original Kaiserling method. In any case in which it is desirable to preserve an unusually large specimen in its original size, gashes should be cut into it, not over 1.5 to 2 inches apart, to allow for penetration of fixative. These may be stitched together later, if desired. After the specimens have been properly fixed, *they should be thoroughly washed in running water for at least from four to six hours.* It is very important that all formaldehyde be removed from the specimens before final preservation. After washing, the specimens are trimmed. All excess fat is removed, and all cut surfaces are resurfaced, by removing a thin layer with a long, sharp knife, to make them smooth and also to remove the upper layer of tissue from which the blood pigments have been extracted. They are then placed in Solution 2.

SOLUTION 2, PRESERVING SOLUTION

Potassium acetate	4,000 gm.
Chloral hydrate	2,000 gm.
Glycerin	4,000 c.c.
Water	36,000 c.c.

Two crocks containing this solution are used. The specimens are allowed to remain in the first crock for twenty-four hours, and are then transferred to the second, and allowed to remain in this until they are to be mounted. When the solution in the first crock becomes dirty, or has an odor of formaldehyde carried over into it, it is discarded, the solution in the second crock is transferred to it, and a fresh solution made in the second crock. For the final mounting fluid, the same formula as given in Solution 2 is used. We have

*The crocks were obtained from the Red Wing Union Stoneware Co., Red Wing, Minnesota.

not found it necessary to use any further chemical to prevent growth of bacteria, moulds, fungi, and so forth. To date we have found no growth in specimens preserved in this solution. However, it may be possible that under different conditions and a different climate some trouble might arise. The addition of a preservative to the mounting solution, in time has a harmful action on the color of the specimens. This applies especially to the use of formol or phenol. With the original Kaiserling formula, arsenious acid, suggested by Delépine, was much better than either of these.

The solution used for filling the jars containing mounted specimens should be perfectly clear. We formerly filtered it through paper, using charcoal. This was a slow and tedious procedure, and often when a considerable amount had been filtered, the paper would break and necessitate refiltering the whole amount. A much simpler method is to use a 10 by 2 inch Berkfeld V filter cylinder, in a detachable nickel-plated metal cylinder, adding powdered animal charcoal, and filtering under a low positive pressure.

There is no new principle involved in this method. The fixing solution is the same as Kaiserling's fixing solution, except for the addition of the chloral hydrate and the lowering of the percentage of the formaldehyde used. The addition of the chloral hydrate apparently prevents the loss of color so common with the original Kaiserling method. Chloral hydrate has been used by Heidenreich, Jores-Köln, Frost, Klotz and Maclachlan, Haythorne, and Klotz and Coburn in their formulas, but all of these authors have left out the chemicals suggested by Kaiserling, and it is these which we believe have an important part in the preservation of the colors. The second solution is the same as Kaiserling's third solution, except that the percentage of potassium acetate and glycerin is reduced, and chloral hydrate is added.

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MODIFIED GRAM STAIN (RUHLAND)*

By Arthur H. Sanford, M.D., Rochester, Minnesota

THERE are many modifications of Gram's differential stain for bacteria. In fact, probably very few bacteriologists use the stain first described by Gram. The essential characteristic of this important procedure is the behavior of the organism when stained with one of the violet dyes: gentian-violet, methyl violet, or crystal violet, then treated with iodine in Gram's iodine solution (one-fifteenth strength Lugol's solution), and decolorized with ethyl alcohol. Acetone decolorization has been very satisfactorily substituted for alcohol by Lyons.[1]

The following formula was given to me fourteen years ago by G. C. Ruhland, at that time director of the laboratories of the Milwaukee Health Department, Milwaukee, Wisconsin, and is now published with his permission. This modification of Gram's stain was entirely original with Dr. Ruhland. I have used it for more than thirteen years in the section on clinical laboratories of the Mayo Clinic, and it is the most satisfactory modification of Gram's stain that I have ever used or seen. Our only modification has been the substitution of acetone for grain alcohol for decolorization. Crystal violet (hexamethyl pararosaniline chloride) can be obtained in the most nearly pure form of any of the violet dyes. Gentian-violet is merely a mixture of penta- and hexamethyl derivatives. Methyl alcohol, the solvent for the dye, is also an excellent mordant. The solution of crystal violet in methyl alcohol is absolutely stabile, a desirable property in any modification of Gram's stain. The safranine counterstain is an excellent contrasting color for the observation of Gram-positive organisms; the Gram-negative organisms are stained a brilliant red.

Formula for Ruhland's Modification of Gram's Stain

SOLUTION A

Crystal violet	2 gm.
Methyl alcohol (special, absolute purity)	100 c.c.

Stain the fixed slide about thirty seconds.
Rinse in tap water.
Then flood the slide with Solution B for thirty seconds or one minute.

SOLUTION B

Gram's Lugol Solution

Iodine	1 gm.
Potassium iodide	2 gm.
Distilled water	300 c.c.

Rinse the slide with tap water and flood it with acetone, C.P. until no more color can be removed; decolorization of a thin preparation takes place instantly.
Counterstain with Solution C.

SOLUTION C

Safranine	1 gm.
Distilled water	100 c.c.

Wash.
Dry.

*From the Section on Clinical Laboratories, Mayo Clinic, Rochester, Minnesota. Presented for publication, September 24, 1924.

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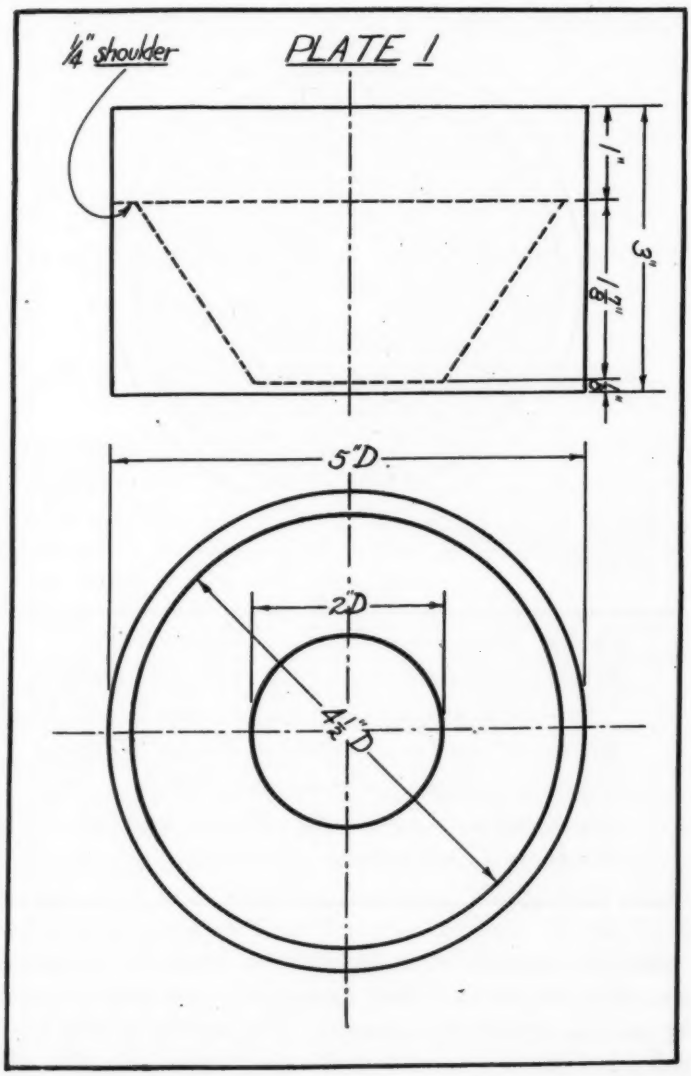
AN IMPROVED SELF-FEEDER FOR RATS*

By Ralph Hoagland, B.Agr., and Oliver P. Clipper, Washington, D. C.

In a previous article,† one of the writers described a self-feeder for rats for use in feeding tests when an accurate record of the food intake was desired. That type of feeder, with slight modifications, has been used in this laboratory during the past three years in feeding tests with more than 2200



*From the Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C.

Received for publication, October 1, 1924.

†Hoagland, R.: A Self-Feeder for Rats, Jour. Lab. and Clin. Med., 1922, vii, 687-689.

rats and it has answered the purpose reasonably well. Experience has shown, however, that it has certain faults, and we have designed a new self-feeder which has proved to be much more satisfactory than the old one.

Scale drawings of the new feeder are shown in Plates I and II. It consists of two parts; viz. (1) Body, as shown in Plate I, and (2), Screen, as shown in

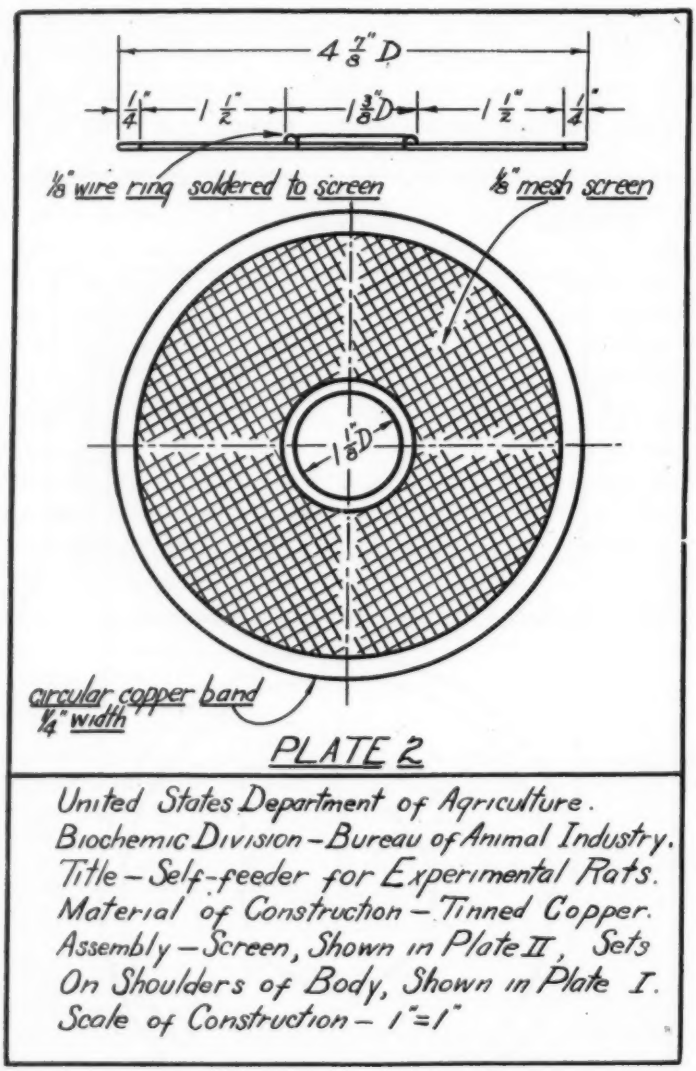

4 7/8" D
1/4"
1 1/2"
1 3/8" D
1 1/2"
1/4"
1/8" wire ring soldered to screen
1/8" mesh screen
1 1/8" D
circular copper band
1/4" width
PLATE 2
United States Department of Agriculture.
Biochemic Division – Bureau of Animal Industry.
Title – Self-feeder for Experimental Rats.
Material of Construction – Tinned Copper.
Assembly – Screen, Shown in Plate II, Sets
On Shoulders of Body, Shown in Plate I.
Scale of Construction – 1"=1"

Plate II. The body consists of a cylinder in which is soldered an inverted truncated cone that serves as a feed receptacle. The body is made of tinned sheet copper and all joints are soldered. The copper at the top and bottom of the cylinder is crimped so as to make the feeder stronger. The screen is made of galvanized iron wire with a copper band around the circumference. The feed hole in the center of the screen is reinforced with a galvanized iron ring which projects above the surface of the screen and serves to prevent feces

from dropping into the feed receptacle. The feeder is simple in construction and substantial.

The feed receptacle or hopper has a maximum capacity of 150 grams, but in practice it has been found best to add approximately 100 grams of feed so as to leave some space between the feed and the screen. This space permits any feed which the rat has carried on to the screen to drop back into the hopper. The feed hole shown in Plate II has proved satisfactory with rats ranging in size from 40 to 200 grams, but a slightly larger opening is required for heavier rats.

The improved self-feeder needs relatively little attention. A rat can easily reach sufficient feed for a period of two or three days without the feeder being attended to, but in practice the feeders are examined daily, except Sunday, and the feed in the hopper is leveled off when necessary. This feeder has proved very satisfactory under a variety of conditions and we intend to replace all of our old type feeders with the new one as rapidly as practicable.

AN IMPROVED DEVICE FOR THE DETERMINATION OF DEXTROSE IN URINE BY FERMENTATION*

By Raymond Szymanowitz, B.S., Newark, N. J.

IN the determination of dextrose in diabetic urine by fermentation, using the Einhorn saccharimeter, dissolved or occluded gases are frequently evolved and collect in the graduated limb of the instrument. This is especially the case when the fermentation is hastened by employing a temperature slightly higher than that of the room.

Lange† found that the small amounts of nitrous and nitric acids in the urine react with urea, amino acids, and ammonia to form nitrogen. A gas so generated, together with the liberation of any entrapped air in the urine, would obviously contribute to the inaccuracy of this method.

To compensate for the solubility of carbon dioxide, the first division of the scale on the graduated limb of the saccharimeter represents a sugar content of slightly less than .25 per cent. For this reason, in the absence of confirmatory tests, air and nitrogen alone might erroneously indicate a sugar content approaching .25 per cent in a dextrose-free urine. In saccharinous urine the results obtained would be higher than the true content. To eliminate the error described herein, the following method and device has been devised.

An Einhorn saccharimeter equipped with a two-way stopcock on its graduated limb, is filled with the urine-yeast solution in the regular way. Upon completion of the fermentation, the saccharimeter is connected with a graduated U-tube, filled with an alkali or any suitable liquid absorbent of carbon dioxide,

*Received for publication, February 5, 1925.
†Berl. klin. Wchnschr., 1921, lviii, 957-9.

through a ground joint as illustrated. The glass tubing carrying the stopcock is of capillary stock and not unlike that employed in general gas analysis.

To free the capillary tubing, as well as the stopcock, of air, the cock is so turned as to permit the escape of a few drops of the contents of the U-tube through the drain tube illustrated. The cock is then turned to a neutral position while the rubber bulb is connected to the shorter limb of the saccharimeter by means of a rubber stopper carrying a small piece of glass tubing.

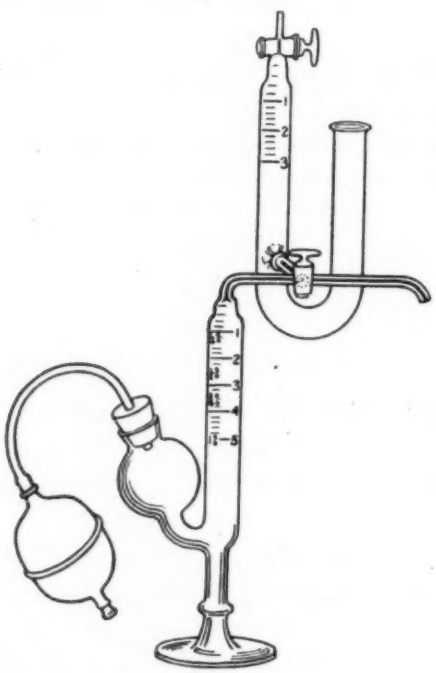


Fig. 1.

By the simultaneous application of gentle pressure to the bulb and proper adjustment of the stopcock, the gases contained in the saccharimeter may be slowly driven into the U-tube where absorption of the carbon dioxide takes place. The comparatively insoluble gases, i. e., nitrogen and oxygen, are found in the graduated limb where they may be read directly as to volume in cubic centimeters. The difference in the readings obtained before and after absorption represents the actual amount of carbon dioxide liberated in the fermentation process in excess of the carbon dioxide in solution in the urine and which was taken into consideration during the calibration of the saccharimeter.

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EDITORIALS

Renal Function In Clinical Medicine

THE kidneys have an abundant blood supply, the renal arteries taking off directly from the abdominal aorta and being quite short. The arterial system leads eventually to the glomeruli of the kidneys where the arterioles break up into large capillary tufts. The vessels leading from the glomeruli are distinctly smaller than the afferent ones. This favors the idea that the blood in passing through the glomerulus loses a large proportion of its water. The arterioles leading from the glomeruli break up again into a second capillary network distributed around the convoluted tubules. This double capillary system is quite unique among the organs of the body and justifies in part the assumption that while water and perhaps other substances pass out through the glomeruli, the excretion of certain other constituents of the urine is a function of the convoluted tubules.

The prevailing theory has it that fluid passes out through the capillary

walls in the glomeruli, through the very thin Bowman's capsule purely by physical processes, and that this transudative fluid contains its constituents in approximately the same concentration as that of the blood plasma. The glomerulus acts as a semipermeable membrane allowing passage to water and most of the crystalloid substances but holding back the colloids. As this very dilute urine passes the cells of the convoluted tubules it is altered by the resorption of some of its constituents through the epithelium and back into the blood stream. Aschoff divides the urine excretory system into three sections, the glomerulus or filtration apparatus, the proximal convoluted tubule or resorption apparatus, and the loop of Henle, which serves as a pressure regulator.

The colloidal chemists explain the resorption of water in the primary tubules on physical grounds. Fischer believes that blood rich in carbon dioxide or venous blood has a tendency to swell or absorb water, while blood poor in carbon dioxide such as arterial blood has a tendency to give up water or shrink. The arterial blood in the glomeruli gives up water readily while the venous blood in the second set of capillaries tends to absorb water.

The resorption of water explains the concentration of urine but it does not explain the alteration in proportion of the various elements. Sodium chloride is concentrated to double the plasma strength, while urea is concentrated about forty times. In explaining this, Cushny divides the constituents of the blood plasma into threshhold bodies and nonthreshhold bodies. Dextrose, chloride and sodium are threshhold bodies and are excreted only when their concentration in the blood exceeds a certain percentage. Threshhold bodies are necessary for vital processes and are retained unless in excess. On the other hand, substances which are of no use to the body, such as urea, are excreted when present even in small amounts and in this way have no appreciable threshhold of excretion.

According to the colloidal theory, substances with threshhold value pass through the glomeruli but are resorbed along with water through the tubular epithelium.

Aschoff maintains that in such a condition as mercury poisoning, mercury is excreted through the glomerulus and it is only during its resorption through the tubules that damage to the latter occurs. Gil-y-Gil has demonstrated that in experimental uranium poisoning, the epithelial cells damaged during resorption acquire a tolerance to the poison. Lethal doses may be subsequently given with impunity, the uranium being excreted in the urine instead of being taken up in the cells. This gives us an insight into one phase of the production of chronic nephritis. The epithelial cells become less permeable, if we may use this term, to the substances which have passed out through the glomeruli and do not resorb them as they should. This is probably true of water as well as of the substances in solution. As a result the concentrating ability of the kidneys becomes damaged.

We have been accustomed to describe parenchymatous nephritis and glomerular nephritis. In the one the damage appears to have been chiefly tubular, in the other glomerular, but both systems are more or less involved in either type and the clinical differentiation is often difficult or impossible. The word nephritis presupposes an infection or inflammation of the kidneys. Nephrosis

is a designation applied to those types of renal pathology in which there is no evidence of infectious origin and in which the cause appears to have been toxic. This applies particularly to those cases with predominant involvement of the tubular epithelium. Parenchymatous nephritis is characterized by degeneration of the epithelium lining the uriniferous tubules with little or no involvement of the glomeruli. The kidney is slightly enlarged, the cortex broadened, and an excess of fat deposit is found in the cells of the tubules. As a general rule there is no cellular infiltration of the connective tissue suggestive of localized infection so that we may consider this condition a nephrosis. This is the type of picture observed in mercuric chloride poisoning.

In acute and chronic glomerulonephritis the gross appearance resembles that of parenchymatous nephritis. The tubules are involved in greater or less degree, the glomeruli are swollen and show an increase in the number of nuclei dependent on multiplication of both endothelial and epithelial elements and in the advanced cases crescentic mass of desquamated cells occupies the capsular space. These are usually inflammatory changes. Glomerular involvement does not undergo such complete recovery as does the parenchymatous type. The vascular tufts become adherent in places to the capsule and destruction of portions of the glomerulus results in partial hyalinization.

The interstitial connective tissue almost invariably undergoes proliferation and it was customary formerly to distinguish a parenchymatous and an interstitial type of nephritis, depending upon the degree of connective tissue formation. Weigert has shown however, that chronic parenchymatous lesions are nearly always associated with interstitial growth. He interprets the connective tissue proliferation as an attempt to fill in the defect caused by the destruction of parenchyma, that is, a replacement fibrosis.

The secondary contracted kidney follows extensive parenchymatous destruction and connective tissue overgrowth and we may look upon this as the end stage of a chronic glomerulonephritis. The scar tissue in the kidney has a patchy distribution.

The primary contracted kidney or red granular kidney differs in many essentials from the types of kidneys just described. In the red granular kidney we observe bands or wedge-shaped areas of fibrous tissue enclosing atrophic tubules and obliterated glomeruli, alternating with active parenchyma. The glomeruli are small, hyaline and surrounded by a thickened capsule. They have suffered total destruction. In other portions of the kidney they are normal. The arterial walls in the red granular kidney are always affected. The elastic layer becomes thickened and the intima undergoes fatty degeneration. These degenerative lesions are undoubtedly a phase of arteriosclerosis and the vascular lesion or arteriosclerosis is probably the chief factor in the production of the primary contracted kidney. The vascular changes are followed by nutritional disturbances which endanger particularly the glomeruli. After their destruction the tubules atrophy from disuse. Thus we see that the renal damage in all forms of nephritis involves both the glomeruli and the tubules, and the variations in the different types are only relative. Even in the arteriosclerotic or

primary contracted kidney both systems are involved. It is apparently only in true nephrosis that the glomeruli remain relatively free from damage.

We can, therefore, readily understand the difficulty of attempting to localize renal injury in the living patient, whether in the tubules or in the glomeruli. It is far safer and more correct to speak of acute or chronic nephritis with or without hypertension, thus describing the renal pathology as a whole. This attitude has been urged particularly by Christian in this country and by Yores and others in Europe.

The fallaciousness of simple qualitative urinalyses is known to all. The value of functional tests is conceded. The interpretation of their significance is on the other hand, not always above question. We no longer think of the salicylic acid test as a measure of the permeability of the glomeruli, the methylene blue test as a measure of the osmotic function of the epithelial cells, and the phloridzin test as a means of determining the secretory ability of the renal epithelium. Even with those tests which are in general use today, it is hazardous to venture concrete hypotheses with regard to their modus operandi in the kidney.

In terms of the preceding discussion we may, however, at a certain risk of accuracy, visualize the reaction of the kidneys to the commonly used functional tests somewhat as follows:

Disease of the kidneys affects the body either by allowing substances to pass out which should be retained, or by retaining substances which ought to pass out. Of the substances which should be retained but do escape, albumin is of chief importance. Of those which should be excreted but are retained, those commonly measured are uric acid, urea, and creatinin. Either the glomerular membrane becomes less permeable to them or they are resorbed in the tubules. The former seems the more likely. These are nonthreshhold substances and normally are not resorbed. Moreover, observations indicate a diminution rather than an increase in the absorptive capacity of the tubules.

Phenolsulphonephthalein is an organic dyestuff toward which the body reacts as it does toward urea. Undoubtedly it is excreted through the glomerular epithelium and normally is not resorbed to any great extent in the tubules. Like urea, in chronic nephritis it probably does not pass as readily through the glomeruli. The reaction of the kidneys to phenolsulphonephthalein is proportionately so similar to their reaction to urea and other nitrogenous substances that the phthalein test has come to be used as a measure of the capacity for excretion of nitrogenous substances.

We cannot at present explain with entire satisfaction the inability of the damaged kidneys to concentrate those crystalloids which do pass the glomerulus. The fluid passing through the glomeruli presumably contains those crystalloids which are not materially obstructed in about the same concentration as in the blood. Failure to concentrate is then presumably due to some change in the tubular epithelium. We have seen that the chronically poisoned epithelium becomes less permeable to certain substances. It is possible that the failure to concentrate substances such as sodium chloride is due to lessened resorptive capacity for water.

We are not so much interested in sodium chloride as an excretory product as we are in the kidneys' ability to concentrate.

It is usually stated that the urea excretion is an index of glomerular function while salt and water excretion is an index of tubular function. The two latter are threshhold bodies. From the foregoing we can see how in a general way this may be true.

We should state parenthetically that the prevailing concept of urinary excretion and concentration, based chiefly on the recent work of Cushny, that concept on which our discussion is based, is not without its weak points. Thus MacNider has demonstrated columnar epithelium in the glomeruli of the 'possum.

Certain generalizations have been made from functional studies, which, while in the main correct, have important exceptions and it is our desire briefly to direct attention to certain of these. It has been stated for example that the examination of a single specimen gives no information regarding the functional capacity of the kidney. It is true that little information is gained from a single urinalysis and that when its specific gravity is low, we have no indication of the functional capacity of the organ. But when the specific gravity is 1020 or above we may be confident that the concentrating ability of the kidney is reasonably good, and that nitrogenous substances are being excreted by the kidney in essentially a normal manner. Thus a single specimen with high specific gravity is of definite value. Of course it is necessary to rule out the presence of sugar.

It has been stated that when the phthalein excretion is high we may be confident that there is no nitrogen retention in the blood. This is not always true. The particular exception is to be found in acute nephritis. The kidney is able to eliminate phenolsulphonephthalein, urea, etc., in normal concentration but the trouble lies in the water excretion which is so greatly impaired that not enough water can pass through to prevent the damming back of urea in the blood.

A high specific gravity does not necessarily indicate normal ability to concentrate urine. We find lack of flexibility in the two-hourly renal test, the variation in specific gravity throughout the day being less than ten points, showing distinct fixation of specific gravity at a high level. This is particularly apt to occur in parenchymatous lesions and in cases of long-standing focal sepsis.

A tendency toward fixation of specific gravity at a high level may appear in normal individuals who from habit have a low water intake. This observation is of help in directing their daily routine in that with a greater fluid intake they develop a normal type of reaction.

No one test is sufficient in all cases and no study of renal function even in the mildest nephritic is sufficiently complete unless all of those tests of proved value have been made and correlated. No single functional test is sufficient for the recognition of early pathology. This is due chiefly to two factors: first, excretion for some substances may be normal while impaired for others, and second, even in the normal there is considerable fluctuation in the response, and multiple checks must, therefore, be applied.

—*W. T. V.*

DR. JOHN A. KOLMER
Philadelphia, Pennsylvania
President, American Society of Clinical Pathologists
1924-1925

The American Society of Clinical Pathologists

Dr. John A. Kolmer, Philadelphia, Pa.
President

Dr. Frederick E. Sondern, New York
1st Vice President

Dr. W. F. Thomson, Beaumont, Texas
2nd Vice President

Dr. Ward Burdick, Denver, Colorado
Seo'y-Treas.

Executive Committee:
Dr. Murray G. Stone, Springfield, Mo.
Dr. Herman Spitz, Nashville, Tenn.
Dr. A. H. Sanford, Rochester, Minn.

Board of Censors:
Dr. Willis P. Butler, Shreveport, La.
Dr. A. H. Schade, Toledo, Ohio
Dr. Ernst A. Victors, San Francisco, Cal.

On to Philadelphia!

History of the American Society of Clinical Pathologists

Introduction.—An organization that is just approaching its third birthday can hardly lay claim to an historical retrospect. So many dramatic events, however, are crowded in the Society's young life that a brief recital of its genesis and growth will probably strike a responsive chord among the members and friends who have watched with interest the meteoric rise of the American Society of Clinical Pathologists.

Predisposing Causes.—The time was ripe for the formation of such an organization. There were many clinical pathologists scattered throughout the medical communities of this country, each more or less isolated from his colleagues in this specialty, trying in his own way to meet the problems that faced him and longing for communion and fellowship with kindred souls for counsel in his difficulties. Above all, it was in the scientific field that he looked for help, guidance or inspiration. Like his confreres in other specialties, he naturally yearned to meet with his peers, at periodical reunions, to discuss the results of the newest research or improved methods in laboratory investigation. None of the existing societies, narrowing their activities to one particular branch of clinical pathology and catering chiefly to college professors, quite satisfied the longings of the practical laboratory man. He desired to meet with men like himself engaged in the professional field, in daily contact with patient and clinician, and confronted with problems similar to his own. In addition to the thirst for knowledge that would aid him in the diagnosis of disease, there was another circumstance that made the formation of a national organization an inevitable outcome. The existence of laboratories, some of them directed by incompetent laymen, advertising their skill and their prices in reputable medical

journals, was a menace that threatened the future continuance of ethical practitioners in this specialty. It was, therefore, a foregone conclusion that sooner or later, a union of the forces striving for a high plane of practice in this field would take place.

Modern historians give little weight to the superman theory ascribing events and occurrences to preexisting causes and external conditions.

Exciting Cause.—So it was with the inception of the American Society of Clinical Pathologists. It so happened that circumstances in one of the western states furnished a favorable nidus for this idea to germinate and evolve to a successful fruition. And now we shall for the nonce forsake the cold impersonal viewpoint and turn to the human interest side of the story of the A. S. C. P.

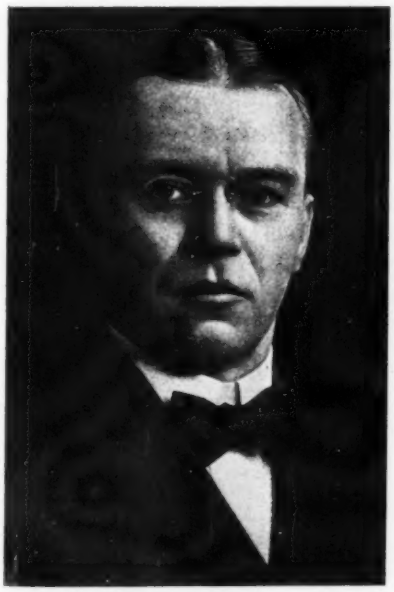
On March 14, 1921, a group of five clinical pathologists, all in active practice of their specialty in the city of Denver, Colorado, met in the laboratory of one of their number to consider the details of a program to be presented before the local County Medical Society on blood chemistry determinations. One of the group who issued the call for this first meeting of the Denver laboratory men had been asked by the Program Committee of the Medical Society to suggest the names of the essayists for the blood chemistry symposium. Fortunately, the esprit de corps of the Denver Clinical Pathologists always has been ideal and their mutual relations the most friendly and cordial. It was but natural, therefore, for them to get together on this occasion and select from their number the speakers who were to present the various angles of blood chemistry in relation to disease. The gathering for this special purpose logically led to the desire for more meetings and thus the Denver Society of Clinical Pathologists was formally launched April 1, 1921.

It was not long before similar friendly relations with colleagues in the neighboring cities of Colorado Springs and Pueblo led to their being invited to participate in these meetings. On June 21, 1921, the name of the organization was changed to the Colorado Society of Clinical Pathologists. All the clinical pathologists of the state were enrolled as members and meetings were held quarterly. At these gatherings, besides the presentation of scientific papers, informal discussions were held on the status of the clinical pathologist and his relation to the clinician. The situation was indeed deplorable. The clinical pathologist was coming to be regarded as a mere technical assistant, not on a par with other specialists. This condescending attitude was greatly fostered by the unseemly advertisements in medical journals tending to make laboratory diagnosis a purely commercial adjunct.

Other Foci of Infection.— Again assuming the historian's viewpoint of cause and effect, it is interesting to note that under similar circumstances a State Society of Clinical Pathologists had been formed in Texas prior to the one in Colorado. Is it that the great open spaces of the West and Southwest where habitations are far apart and the herd instinct more pronounced that conditions favor the spirit of neighborliness more than in the congested East? It is idle to speculate on the etiology or lapse into generalization which may prove illogical for lo, about the same time we find Ohio organizing a State

DR. PHILIP HILLKOWITZ
Denver, Colorado
Past President, 1922-1923

DR. WM. CARPENTER MACCARTY
Rochester, Minnesota
Past President, 1923-1924



DR. FREDERICK E. SONDERN
New York City, N. Y.
First Vice-President

DR. WARD BURDICK
Denver, Colorado
Secretary-Treasurer

Pathological Society. Friendly correspondence with these state organizations cheered each one on in its work.

Period of Polemic.—With the encouragement of the sister society of Texas, the officers of the young Colorado Society of Clinical Pathologists launched their famous campaign against the advertisement of laboratories in medical journals. With the enthusiasm characteristic of youth, numerous conferences were held and plans laid for fighting the evil. Courteous letters of protest were sent to the trustees of the American Medical Association calling attention to the unseemly advertisements in the Journal as unworthy of the good character of the official organ of the medical profession of America. Resolutions decrying the acceptance of advertisements from commercial laboratories by medical journals were drafted and submitted before the Medical Society of the City and County of Denver which passed it unanimously, other medical societies later following the example.

Letters of mild protest were sent to honorary consultants whose names figured in the advertisements of a laboratory on the "chain of stores" plan, pointing out the anomaly of their position as teachers in medical schools lending their prestige to a commercial concern. All this formed a lively chapter in the career of the newly organized state society, the battle for the right spurring it on to greater efforts, the resistance of the opposition only stimulating the quicker coursing of the blood.

Preliminaries to Organization.—The need of a national body to take up the fight was sorely felt. It transcended, for a while, the yearning for the scientific purpose of such an organization. How can a few humble and obscure pathologists, in a provincial town in the far away West set the machinery in motion to get a national organization started? Yet the matter was quite simple. The conditions were ripe. The determining factors were all there. Chance and a little initiative got the wheels a-going. Late in 1921, a letter and questionnaire were sent out on the letterheads of the Colorado Society to all the colleagues listed in the American Medical Association Directory under the designation CP with an asterisk, signifying that they limited their practice to Clinical Pathology. The culling of the names, in itself a task, was distributed among the members and in a trice the list was complete. A copy of the circular may be of historical interest.

"The formation of societies of clinical pathologists in various cities and states has naturally led to a desire for the creation of a national organization.

"With this object in view we are addressing a circular letter and questionnaire to all physicians who specialize in this branch of medicine.

"The necessity for an organization of this kind is very urgent in view of the strong tendency to commercialize our specialty and to degrade the pathologist to a mere technician.

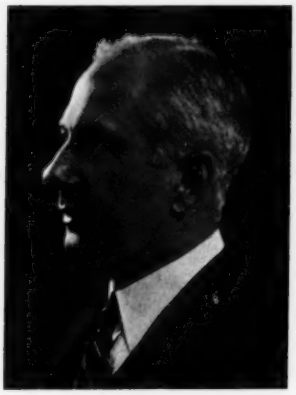
"The scope of the proposed organization would be to advance the scientific progress of Clinical Pathologists and to maintain the status of the Clinical Pathologist as a consultant in medicine on an equal plane with that of the internist or surgeon.

"It is proposed to form this organization at the next meeting of the American Medical Association at St. Louis, where problems relating to our specialty may be discussed personally and in detail.

"Any suggestions from you will be greatly appreciated."

DR. H. J. CORPER
Denver, Colorado
Member of Publication Committee

DR. F. M. HUNTOON
Glenolden, Pennsylvania
Member of Committee on Standardization of Laboratory Reagents



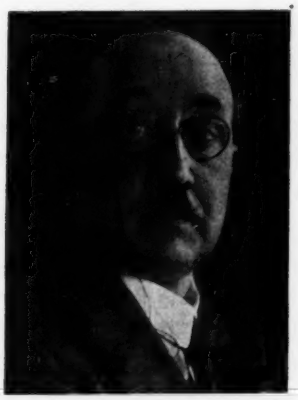
DR. THADDEUS WALKER
Detroit, Michigan
Member of Standardization Committee

DR. RUTH GILBERT
Albany, New York
Member of Standardization Committee

DR. J. H. BLACK
Dallas, Texas
Member of Committee on Standardization of Laboratory Reagents

DR. A. V. ST. GEORGE
New York City, New York
Member of Standardization Committee

DR. GEORGE IVES
St. Louis, Missouri
Member of Publication Committee



DR. WM. C. EXTON
Newark, New Jersey
Member of Publication Committee

Attached was a questionnaire inquiring among other things whether the addressee would join a national society of Clinical Pathologists and whether he would attend the session of the A. M. A. at St. Louis where the proposed society would be organized.

To the four hundred and fifty letters that were sent out there came back one hundred and seventy-five replies, the great majority of which fervently advocated the formation of such a society and many of them promising to attend the convention and be ready for the call.

Heartened by the extraordinarily favorable response, steps were soon taken to issue a call for the convention. It was deemed advisable to make the time and place coincident with the meeting of the American Medical Association in order to insure a good attendance. Dr. George Ives of St. Louis having been among the first to respond to the questionnaire was asked to act as a Committee of Local Arrangements, and right loyally did he execute the duties allotted to him. A commodious room in the Missouri Baptist Hospital was, through his efforts, assigned as a meeting place for the clinical pathologists. At the proper time announcements of the forthcoming convention were sent to all the colleagues on our mailing list.

The First Annual Convention.—The fateful day of the meeting was drawing near. Arrangements had all been completed. It was with some fear and trepidation that the signers of the call awaited the portentous event. On Monday, May 22, 1922, at eight P.M., about forty men and a few ladies were assembled at the meeting place awaiting developments. Very few of those present were personally acquainted with one another and the atmosphere was somewhat tense and expectant. The meeting was called to order by Dr. Ives. In the minutes of the first convention are found his prophetic introductory remarks. "I have assumed the honor of calling to order what is destined to be the first successful national meeting of Clinical Pathologists."

In accordance with parliamentary procedure, he nominated a temporary chairman and on the latter's assumption of the chair, he also nominated a temporary secretary. The courtesy of these two offices was accorded to the Colorado delegates—Dr. Philip Hillkowitz and Dr. Ward Burdick, who had issued the call.

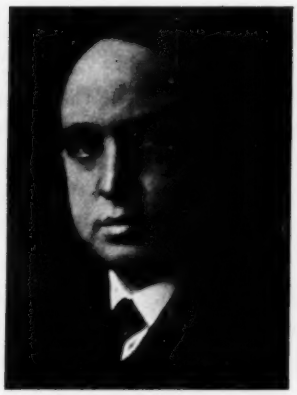
Parturient Pains.—After a brief exposition by the chairman on the purpose of the convention, a general discussion took place on the meaning of the term "clinical pathologist" which brought forth a variety of views, but helped to clear the atmosphere and pave the way for definite organization.

On motion of Dr. MacCarty of Rochester, a committee of five was appointed to carry out the purpose of the convention and submit a constitution and by-laws for consideration. After further extended discussion on the part of those present on the relation of the pathologist to the clinician, the session adjourned until the following morning. The differences of opinion on the matters under consideration left the delegates with an uneasy feeling and some misgivings as to the future fate of the proposal for a national organization.

The Birth.—The next morning was ushered in with a flood of sunshine. The visitors had rested from their travel and were in fine humor. Promptly

DR. HERMAN SPITZ
Nashville, Tennessee
Member of Executive Committee

DR. MURRAY C. STONE
Springfield, Missouri
Member of Executive Committee



DR. A. H. SANFORD
Rochester, Minnesota
Member of Executive Committee

DR. W. F. THOMSON
Beaumont, Texas
Second Vice-President

DR. ERNST A. VICTORS
San Francisco, California
Member of Board of Censors

DR. A. H. SCHADE
Toledo, Ohio
Member of Board of Censors

DR. WILLIS P. BUTLER
Shreveport, Louisiana
Member of Board of Censors

at eleven o'clock, the temporary chairman opened the meeting and immediately called for the report of the Committee on Constitution and By-Laws.

In the brief interval between the sessions, the committee would have had to work all night drafting the instrument but the machinery of the organization had been so well oiled that typewritten copies of a constitution and by-laws were in the hands of the committee immediately after their appointment and met with their approval so that but few alternations were made. The members present seemed to think likewise, for without any discussion the acceptance of the constitution and by-laws was carried unanimously. The temporary officers were elected president and secretary, respectively, for the ensuing year; Dr. William C. MacCarty, of Rochester, Minnesota, was chosen as first vice-president and Dr. H. R. Brown, of Rochester, New York, as second vice-president. To lend auspicious augury to the new organization a program committee appointed by the Chair hastily arranged from the talent present an extemporaneous scientific session for the afternoon gathering. Dr. Ives spoke on "Streptococcus Veridans Endocarditis" and Dr. MacCarty on "The Diagnostic Efficiency of the Average Clinician with Reference to Breast Tumors," both addresses being liberally discussed.

By this time the members had become well acquainted with each other. A feeling of fellowship was engendered. At the close of the meeting everyone felt elated at the success achieved and filled with high hopes for the future of the American Society of Clinical Pathologists.

Consolidation of Organization.—The year of 1922-23 was devoted to building up and strengthening the organization. The number of charter members at the close of the first annual convention was one hundred and forty-five. With the aid of these members and through the efforts of the Secretary's office, applications for membership rapidly increased.

Education of the Profession.—Considerable efforts were put forth to elevate the status of the clinical pathologist. The trustees of the American Medical Association were again appealed to in our campaign against laboratory advertising. A communication protesting against the action of the Advertising Committee of the A. M. A. in permitting the insertion of objectionable advertisements was published in the Journal and helped to arouse a more sympathetic attitude of the medical profession toward the clinical pathologist.

Our members were kept apprised of our progress by means of Bulletins which carried detailed accounts of our fight for the Ethical Practice of Clinical Pathology together with other items of interest.

The morale of the Clinical Pathologists throughout the country was thereby raised to an incalculable degree. They felt as never before that they were specialists in an important branch of medicine, consultants, on a par with the internist and the surgeon.

As the year rolled on the enthusiasm of the members grew apace and they looked forward with pleasant anticipation to the next annual meeting.

Second Annual Convention.—Again it was deemed advisable to hold the convention at the same time and place as that of the American Medical Association scheduled for that year at San Francisco. On account of the great

distance from Eastern centers no large attendance was anticipated. Nevertheless over seventy members registered at the second annual convention of the American Society of Clinical Pathologists which was held June 25, and 26, 1923. The scientific program was very good, the chief subject of interest and discussion being the technic of the Wassermann reaction. Considerable interchange of opinion took place on the economic aspects of the practice of clinical pathology. This was occasioned, to some extent by the presence of several men affiliated with commercial laboratories who objected to the attitude of the American Society of Clinical Pathologists toward advertising. The Society, however, by a practically unanimous vote registered its approval of the code of ethics laid down in its by-laws.

During the course of the meeting the trustees of the American Medical Association invited a committee from the American Society of Clinical Pathologists to give their view of laboratory advertising which was accordingly presented to them in the proper light.

As a result of the campaign waged by the American Society of Clinical Pathologists, laboratory advertisements though not entirely eliminated have been censored to the extent that no fees are published or undue claims made.

The membership during the second year continued to grow. Clinical pathologists all over the country, recognizing the valuable services rendered their cause by the society hastened to enroll under its banner.

In the election of new officers, Dr. Wm. C. MacCarty of Rochester, Minnesota, was chosen president, Dr. John A. Kolmer, first vice-president, Dr. George Ives, St. Louis, Missouri, second vice-president and Dr. Ward Burdick was reelected secretary-treasurer.

The incoming president invited the society to meet the next year in Rochester, Minnesota, as guests of the Mayo Clinic. The invitation was heartily accepted.

Third Annual Convention.—The central location, the large and interesting scientific program, the opportunity to see the extensive laboratories of the Mayo Clinic, all combined to bring out a large number of members and visitors at the Rochester meeting which was held June 5, to 7, 1924. By this time many of the members were well known to one another and cordial greetings of good fellowship were exchanged. The hospitality of the Mayos in offering the members all the facilities of their wonderful plant was deeply appreciated by all present at the meeting.

The papers presented were of a high order and the round table discussions by experts in their respective fields were very instructive.

Nothing but science held sway at the third annual meeting. Business matters and economic topics which loomed large at San Francisco were relegated to the background. It was clearly the intention of the committee of arrangements to demonstrate that the American Society of Clinical Pathologists is primarily a scientific society and that the other aspects of membership in this organization, though likewise desirable, are secondary. All the visitors went away profoundly impressed and highly elated at the high scientific level at-

tained by the society and correspondingly proud of the privilege of belonging to such an organization.

Official Organs.—Tentative arrangements had been made prior to the Rochester meeting for the JOURNAL OF LABORATORY AND CLINICAL MEDICINE to publish matters of interest to our members. During the convention the opportunity was presented through personal interviews with the editor and the publisher of the Journal to make this excellent periodical our official organ. All the papers presented at the meeting together with the transactions were accordingly published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

Officers Elected at the Third Annual Convention.—At the Rochester gathering, the society elected Dr. John A. Kolmer, of Philadelphia, Professor of Clinical Pathology at the University of Pennsylvania, President; Dr. Frederick E. Sondern of New York City, a pioneer in Clinical Pathology, first vice-president; Dr. W. F. Thomson, Beaumont, Texas, second vice-president, and Dr. Ward Burdick was reelected secretary-treasurer.

The Present.—The approach of the Fourth Annual Convention finds the American Society of Clinical Pathologists in a flourishing and healthy state. The perilous period of infancy when the mortality is highest has been safely passed over; the seasons of storm and stress when it was battling for recognition as a dignified specialty, are also left behind. The society is now a lusty youth full of enthusiasm and filled with ideals for a useful future. Its membership now exceeding three hundred and representing the best men in this field in all parts of the United States is devoted to the cause of helping humanity by advancing the cause of scientific medicine through the aid of clinical pathology.

The Administration of the American Society of Clinical Pathologists

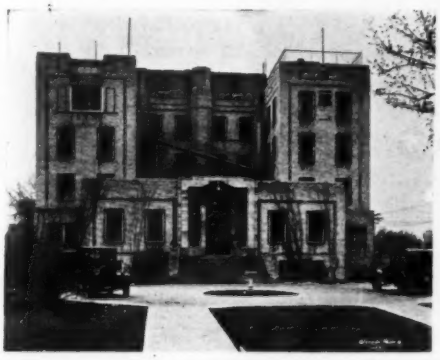
From a portable typewriter in a corner of a crowded laboratory in a physicians' office building to a full complement of office equipment in a suite of commodious quarters in a modern hospital—such is the story of the rapid rise of the secretariat of the American Society of Clinical Pathologists. In the early days of the society—if it be proper to indulge in reminiscences of a three-year-old organization—all the business of the new fledgeling was conducted in the interim of making laboratory examinations with a microscope in front and an incubator in the background. The correspondence was pounded out on a small portable typewriter, and records kept in paper files.

The phenomenal growth of membership and enormous increase in correspondence soon necessitated having clerical help, at first only during periods of stress but later on half time basis. The need for better equipment and more office space was soon apparent. Fortunately the Children's Hospital, with which our secretary is connected as pathologist, has extended to the society the free use of very roomy quarters in this institution adjoining the clinical laboratory.

In this office is installed the usual armamentarium of a modern business office including a set of files which hold, besides the correspondence, an elaborate

series of card indexes covering all the activities of the American Society of Clinical Pathologists. Every member is listed both alphabetically and geographically with data pertaining to his membership.

The office of the American Society of Clinical Pathologists has developed into an information bureau and clearing house. Its activities are manifold. Aside from keeping in constant touch with the membership on the progress of the organization, it answers the many inquiries that come in on society matters. It receives and answers correspondence from hospitals and colleges



Children's Hospital, Denver, Colorado, wherein is located the office of the Secretary of the American Society of Clinical Pathologists.

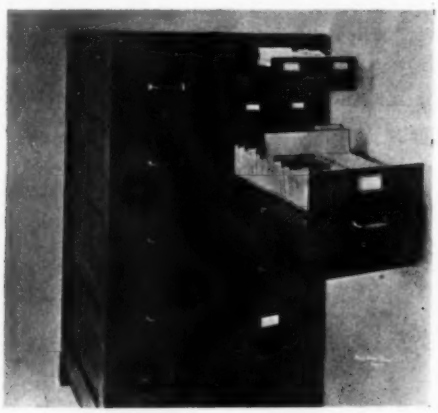
Secretarial Quarters. The office is furnished with all necessary business appliances for conducting the affairs of a national organization.

Miss Marie Boes, Assistant to the Secretary

View of the offices of the American Society of Clinical Pathologists. Across the corridor are the laboratories of the Children's Hospital of Denver, Colorado.

regarding available clinical pathologists and teachers in pathology, bacteriology and allied subjects. It likewise puts the clinical pathologist seeking a new location in touch with the hospital offering the prospect of a position. Much of the work of the publication committee is carried on in this office, also correspondence relative to clinical pathologists seeking membership, the arrangement of our annual program, matters consequent to our annual conventions, conducting detail work of various committees such as the Committee on Standardization of Laboratories, etc. With each year the functions of the

secretariat have been broadened so that at the present time a full time assistant to the secretary has been found necessary to keep up with the increasing volume of office work.



A close-up of the filing system. There is a folder for the correspondence of and the data on every member of the Society; also ledger cards with alphabetical and geographical classification.

It is indeed a source of gratification to observe the gigantic strides that have been made by the American Society of Clinical Pathologists as reflected in the scene of busy activity in the offices of the administration.

Program of the Fourth Annual Meeting of the American Society of Clinical Pathologists

Benjamin Franklin Hotel, 9th and Chestnut Streets, Philadelphia, Pennsylvania.

WEDNESDAY, MAY 20, 9 A.M.

Call to Order—Short Business Session

Scientific Program

The Preparation of Solutions of Dextrose for Intravenous Administration, by Dr. Ralph G. Stillman, New York City, N. Y.

Utilization of Carbohydrates, by Dr. W. G. Karr, Philadelphia, Pennsylvania (by invitation).

Diagnostic Value of Spinal Fluid Sugar Content, by Dr. W. Parker Stowe, Rochester, New York.

Alveolar CO_2 Tension and Acetone in the Expired Air in Acidosis, by Dr. Paul Roth, Battle Creek, Michigan.

The Effect of Varied Carbohydrate Food in the Blood Sugar Concentration, by Dr. Leon Jonas, Philadelphia, Pa. (by invitation).

WEDNESDAY, MAY 20, 2 P.M.

Aids to Urinalysis in a Large Hospital, by Dr. C. Pons and Dr. E. B. Krumbhaar, Philadelphia, Pennsylvania (by invitation).

A New System of Duplicating and Placing Laboratory Reports in the History, by Dr. Frank W. Hartman, Detroit, Michigan.

Value of Method of Keeping Records of Tissue Examinations, by Dr. L. A. Turley, Norman, Oklahoma.

A New Type of Instrument for the Estimation of Hemoglobin, by Dr. C. E. Roderick, Battle Creek, Michigan.

Some Useful Apparatus, by Dr. Max Shaweker, Dover, Ohio.

The Organization of a Metabolism Laboratory, by Dr. William H. Stoner, Philadelphia, Pennsylvania (by invitation).

Value of the Leucocyte Fragility Test in the Prognosis of Pneumonia, by Dr. C. Pons and Dr. E. P. Ward, Philadelphia, Pennsylvania. (By invitation).

The Value of the Ieteric Index in Differentiating Anemia, by Dr. A. V. St. George, and Dr. A. L. Brown, New York City.

A Plea for a Standard Method of Determining and Reporting Hemoglobin Values, by Dr. E. Clarence Rice, Washington, D. C.

Myocardial and Endocardial Changes in Cardiac Defects, by Dr. Maude E. Abbott, Philadelphia, Pennsylvania.

The Benjamin Franklin, Philadelphia
Headquarters, American Society of Clinical Pathologists
May 20, 21, 22, 23, 1925

THURSDAY, MAY 21, 9 A.M.

Autopsy Report of Two Cases of Thymic Death During Surgical Operations, by Dr. Jessie W. Fisher, Middletown, Connecticut.

Some Enzyme Studies with Desiccated Gonadal Tissue, by Dr. Herman Sharlit and Dr. William G. Lyle, New York City, N. Y.

A Comparison of Enzyme Actions of Tumors and Normal Tissues, by Dr. K. George Falk and Helen Miller Noyes, New York City, N. Y., (by invitation).

A Double Hydrogen Electrode System for the Determination of Hydrogen-Ion Concentration, by Dr. George H. Meeker and Mr. Bernard Oser, Philadelphia, Pennsylvania, (by invitation).

Observations on the Dick Test, Toxin Immunization and Serum Treatment of Scarlet Fever, by Dr. John A. Murphy, Philadelphia, Pennsylvania (by invitation).

Pneumococcus Antibody Solution, by Dr. Frank M. Huntoon, Glenolden, Pennsylvania.

Anisocytosis and Increased Red Blood Cell Volume with Little or no Anemia, (Lantern Slides), by Dr. Mortimer Warren, Portland, Maine.

THURSDAY, MAY 21, 2 P.M.

Normal and Pathologic Basal Metabolic Rate in Obesity, by Dr. Horry M. Jones, Chicago, Illinois.

Studies on Blood Cultures with Special Reference to the "Massive" Method, by Dr. Herbert Fox and Dr. William G. Leaman, Philadelphia, Pennsylvania.

A Comparative Study of Liver Functional Tests, by Dr. A. I. Rubenstone and Dr. Louis Tuft, Philadelphia, Pennsylvania.

The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method, by Dr. B. S. Parks, Philadelphia, Pennsylvania (by invitation).

Studies on the Bacteriology of the Urine in Cooperation with Catheterization of the Ureters, by Dr. Robert A. Keilty, Danville, Pennsylvania.

Study of Cases of Acute Leukemia and Acute Mononucleosis, by Dr. B. L. Crawford, and Dr. Harold W. Jones, Philadelphia, Pennsylvania.

Blood Counts in Mississippi, by Dr. Leon S. Lippencott, Vicksburg, Mississippi.

THURSDAY, MAY 21, 7 P.M.

Reaction After Typhoid Vaccination, by Dr. Henry J. Nichols, Washington, D. C.

The Measurement of Cloudiness in Liquids, by Dr. William G. Exton, Newark, New Jersey.

Studies in Embalming Fluids in Relation to Gross and Histological Tissue Examinations, by Dr. John A. Kolmer and Dr. Fred Boerner, Philadelphia, Pennsylvania.

The Specific Inflammatory Reaction of Immunized Animals (Arthus Phenomenon), by Dr. Eugene L. Opie, Philadelphia, Pennsylvania (by invitation).

Methods of Staining Tubercle Bacilli, by Dr. H. J. Corper, Denver, Colorado.

FRIDAY, MAY 22, 9 A.M.

Sedimentation Rate of Erythrocytes, by Dr. H. N. Cooper, Watertown, New York.

Standardization of Tuberculin, by Dr. Joseph D. Aronson, Philadelphia, Pennsylvania (by invitation).

Bronchial Spirochetosis, by Dr. Thomas L. Ramsey, Toledo, Ohio.

Etiological Studies in Psoriasis, by Miss Mary Marcus, Philadelphia, Pennsylvania (by invitation).

Squamous Cell Carcinoma of the Gall Bladder, by Dr. Frank W. Hartman, Dr. W. E. Johnson, Detroit, Michigan.

The Technic of the Practical Application of the Pathogen Selective Cultural Method, by Dr. A. I. Rubenstone, Philadelphia, Pennsylvania.

FRIDAY, MAY 22, 2 P.M.

A System of Laboratory Approval Proposed to the American Society of Clinical Pathologists, by Dr. Frederick E. Sondern for the Committee on Standardization.

Discussion to be opened by Dr. Ruth Gilbert, Dr. C. Y. White (by invitation) and Dr. S. R. Haythorn.

Open Discussion

How Can We Best Promote the Objects Contained in Article II of Our Constitution? By Dr. Herman Spitz, Nashville, Tennessee.

Suggested Method to be Followed in Developing a Standardized Course for Medical Technicians, by Dr. Walter E. King, Detroit, Michigan.

FRIDAY, MAY 22, 7 P.M.

Annual Dinner in Ball Room of the Benjamin Franklin Hotel.

Presidential Address

The Functions of a Hospital, by Dr. George H. Meeker, Dean of the Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

SATURDAY, MAY 23, 9 A.M.

Business Session

SATURDAY, MAY 23, 2 P.M.

The Clinical Significance of Anticomplementary Serum, and Spinal Fluids in the Wassermann Reaction, (Kolmer Modification), by Dr. A. H. Sanford, Rochester, Minnesota.

A Standardized Wassermann Report, by Dr. A. J. Casselman, Camden, New Jersey.

The Present Status of the Kolmer Complement-Fixation Test for Syphilis as Determined by a Comparison with Numerous Other Methods, by Dr. Robert A. Kilduffe, Atlantic City, New Jersey.

Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, by Dr. Robert G. Owen, Detroit, Michigan.

Kahn's Precipitation Reaction as Compared to Kolmer's Complement-Fixation Test, by Dr. A. S. Giordano, South Bend, Indiana.

A Clinical Study of the Kolmer and Kahn Reactions in Syphilis, by Dr. R. L. Kelly, Louisville, Kentucky (by invitation).

The Meinecke Reaction as Compared with the Wassermann in One Thousand Specimens of Blood Sera. (Lantern Slides), by Dr. A. M. P. Saunders, Dunning, Illinois.

Scientific Exhibit

Microscopic and Other Specimens Relating to Tropical Medical Problems, by Dr. J. M. Feder, Santo Tomas Hospital, Panama.

Commercial Exhibits

The commercial exhibits are an added attraction to the Meeting of the American Society of Clinical Pathologists. Many of the leading laboratory houses will display a large line of new and up to the minute laboratory equipment.

Every form of apparatus will be on display in those exhibits that goes to make a complete diagnostic clinical laboratory. The leading medical publishers will exhibit a complete line of laboratory books, as well as a large number of books on allied subjects.

All of the exhibits are being planned to serve the convenience of the clinical pathologist, and it will be to his advantage to visit them often.

The Program speaks for itself and is a sufficient lure for all Clinical Pathologists to temporarily forsake their laboratories and participate in this intellectual and scientific treat.

All members should make plans immediately for attending our next meeting. The Secretary will gladly make hotel reservations for the visitors and their families. Please communicate at once with Dr. Ward Burdick, Children's Hospital, Denver, Colorado.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*Canned Foods in Relation to Health**

IN his Milroy lectures for 1923, published as a volume of the Cambridge Public Health series, William G. Savage undertakes an exhaustive study and criticism of the methods of preparation, the possibilities of contamination, and other potential sources of danger in connection with canned goods. Written originally as a lecture before the Royal College of Surgeons, it has an easy style which makes the volume very pleasant reading. Savage has undertaken a thorough study of canning methods both in England and in the United States and has covered the literature thoroughly. Among other points, acid foods kept in a tin for a long time will dissolve sufficiently large amounts of tin to become of pathologic significance.

That canning is not 100 per cent efficient is indicated by the observation that, in 1920, the wastage of canned meat alone was 1,354,272 pounds. A great deal of this is due to the fact that bacteria are not entirely destroyed in the process of canning. In investigations of different classes of foods by several observers no class of products was found to be always sterile, the percentage varying from 18 per cent not sterile for unsweetened milk to 84 per cent not sterile for Crustacea and 100 per cent for sweetened milk. Practically all bacteria except resistant spore bearers are destroyed. These remain viable and cause no further damage provided there is no unusual access of air.

The two most important factors in spoilage are conditions within the tin controlling the availability of free oxygen, and the initial number of organisms present. The essential importance of absolutely air-tight retainers is not that outside bacteria might gain access, but that the admission of air may render conditions suitable for the multiplication of those bacteria which are already present. Even obligate anaerobes may be stimulated to become actively decomposing organisms by the introduction of air.

Even in the absence of bacteria, that is, in properly sealed cans, with sound contents, certain chemical changes occur with aging, known to the trade as maturation. In the case of marine products this is especially desirable and improves the product. There is no evidence so far that maturation in sound tins results in the formation of any substance dangerous to health.

Recent studies of botulism and the effect of canning upon vitamines are discussed in detail.

The volume constitutes a distinct contribution to the science and the industry of canning and will be of particular value to students of public health and of industrial bacteriology.

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*Canned Foods in Relation to Health. (Milroy Lectures, 1923.) By William G. Savage, B.S., M.D., (Lond.) D.P.H. Cloth. Pp. 146. Price 8s 6d. University Press, Cambridge, 1923.